SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: High-risk Human Papillomavirus mRNA Detection Kit

Device Trade Name: APTIMA® HPV 16 18/45 Genotype Assay

Device Procode: OYB

Applicant's Name and Address:

Gen-Probe Incorporated
10210 Genetic Center Drive
San Diego, CA 92121-4362

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P120007

Date of FDA Notice of Approval: October 12, 2012

Expedited: Not applicable

II. INDICATIONS FOR USE

APTIMA HPV 16 18/45 Genotype Assay Indications for Use:

The APTIMA HPV 16 18/45 Genotype Assay is an *in vitro* nucleic acid amplification test for the qualitative detection of E6/E7 viral messenger RNA (mRNA) of human papillomavirus (HPV) types 16, 18, and 45 in cervical specimens from women with APTIMA HPV Assay positive results. The APTIMA HPV 16 18/45 Genotype Assay can differentiate HPV 16 from HPV 18 and/ or HPV 45, but does not differentiate between HPV 18 and HPV 45. Cervical specimens in ThinPrep Pap Test vials containing PreservCyt Solution and collected with broom-type or cytobrush/spatula collection devices* may be tested with the APTIMA HPV 16 18/45 Genotype Assay. The assay is used with the TIGRIS DTS System.

The use of the test is indicated:

1. In patients 21 years and older with atypical squamous cells of undetermined significance (ASC-US) cervical cytology results, the APTIMA HPV 16 18/45 Genotype Assay can be used to test samples from women with APTIMA HPV Assay positive results to assess the presence or absence of high-risk HPV genotypes 16, 18, and/or 45. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management. The results of this test are not intended to prevent women from proceeding to colposcopy.
2. In women 30 years and older, the APTIMA HPV 16 18/45 Genotype Assay can be used to test samples from women with APTIMA HPV Assay positive results. The assay results will be used in combination with cervical cytology to assess the presence or absence of high-risk HPV genotypes 16, 18, and/or 45. This information, together with the physician's

assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.

* Broom-type device (e.g., Wallach Pipette), or endocervical brush/spatula.

III. CONTRAINDICATIONS

None.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the APTIMA HPV 16 18/45 Genotype Assay labeling.

V. DEVICE DESCRIPTION

The APTIMA HPV 16 18/45 Genotype Assay involves three main steps, which take place in a single tube: target capture; target amplification by Transcription-Mediated Amplification (TMA); and detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA). The assay incorporates an Internal Control (IC) to monitor nucleic acid capture, amplification, and detection, as well as operator or instrument error.

Specimens are transferred to a tube containing specimen transport media (STM) that lyses the cells, releases the mRNA, and protects it from degradation during storage. When the APTIMA HPV 16 18/45 Genotype Assay is performed, the target mRNA is isolated from the specimen by use of capture oligomers that are linked to magnetic microparticles. The capture oligomers contain sequences complementary to specific regions of the HPV mRNA target molecules as well as a string of deoxyadenosine residues. During the hybridization step, the sequence-specific regions of the capture oligomers bind to specific regions of the HPV mRNA target molecule. The capture oligomer-target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles, including the captured HPV mRNA target molecules bound to them, are pulled to the side of the reaction tube using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification inhibitors.

After target capture is complete, the HPV mRNA is amplified using TMA, which is a transcription-based nucleic acid amplification method that utilizes two enzymes, MMLV reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy of the target mRNA sequence containing a promoter sequence for T7 RNA polymerase. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template.

Detection of the amplicon is achieved by HPA using single-stranded nucleic acid probes with chemiluminescent labels that are complementary to the amplicon. The labeled nucleic acid probes hybridize specifically to the amplicon. The Selection Reagent differentiates between hybridized and unhybridized probes by inactivating the label on the unhybridized probes. During the detection step, light emitted from the labeled RNA-DNA hybrids is measured as photon signals called Relative Light Units (RLU) in a luminometer. Final assay results are interpreted based on the analyte signal-to-cutoff (S/CO) ratio. IC is added to each reaction via the Target Capture Reagent. The IC monitors the target capture, amplification, and detection steps of the assay. The Dual Kinetic Assay (DKA) is the method used to differentiate the HPV signals and the IC signal.

IC and HPV 16 amplicon are detected by probes with rapid light-emission kinetics (flasher). The IC signal in each reaction is discriminated from the HPV 16 signal by the magnitude of the light emission. Amplicons specific to HPV 18 and 45 are detected using probes with relatively slower kinetics of light emission (glower).

Additional details can be found in the operator's manual for the device.

Test Interpretation

Test results are automatically determined by the assay software. A test result may be negative for both HPV 16 and HPV 18/45, negative for HPV 16 and positive for HPV 18/45, positive for HPV 16 and negative for HPV 18/45, positive for both HPV 16 and HPV 18/45, or invalid as determined by the RLU and signal-to-cutoff (S/CO) ratios as described in the table below. A test result may also be invalid due to other parameters (e.g., abnormal curve shape) being outside the normal expected ranges. Invalid test results should be repeated.

APTIMA HPV 16 18/45 Genotype Assay Result	**Criteria**
Negative - 16 **Negative - 18/45**	*IC/HPV 16 RLU ≥ IC Cutoff and* *HPV 16 S/CO < 1.00 and* *HPV 18/45 S/CO < 1.00*
Negative - 16 **Positive - 18/45**	*HPV 16 S/CO < 1.00 and HPV 18/45 S/CO ≥ 1.00 and* *HPV 18/45 RLU ≤ 3,000,000*
Positive - 16 **Negative - 18/45**	*HPV 16 S/CO ≥ 1.00 and* *IC/HPV 16 RLU ≤ 4,000,000 and* *HPV 18/45 S/CO < 1.00*
Positive - 16 **Positive - 18/45**	*HPV 16 S/CO ≥ 1.00 and* *IC/HPV 16 RLU ≤ 4,000,000 and* *HPV 18/45 S/CO ≥ 1.00 and* *HPV 18/45 RLU ≤ 3,000,000*
Invalid	*HPV 16 S/CO < 1.00 and HPV 18/45 S/CO < 1.00 and* *IC/HPV 16 RLU < IC cutoff* *Or IC/HPV 16 RLU > 4,000,000* *Or HPV 18/45 RLU > 3,000,000*

NOTE: Results from user-provided external quality control samples must be monitored and assessed by laboratory personnel per laboratory procedures.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

Two alternatives for the genotyping of high-risk HPV are currently approved in the United States. Both alternative devices detect HPV DNA instead HPV mRNA and do not include HPV genotype 45, although the risk profile and performance with this new genotype included is similar. Each HPV detection method has its own advantages and disadvantages.

A patient should fully discuss these alternatives with her physician to select the screening method(s) that best meets expectations and her lifestyle.

VII. MARKETING HISTORY

The APTIMA HPV 16 18/45 Genotype Assay was CE-marked on July 6, 2012 and registered in the countries listed below. As of September 17, 2012, the APTIMA HPV 16 18/45 Genotype Assay has not been distributed for commercial use.

- Austria
- Belgium
- Bulgaria
- Czech Republic
- Finland
- France
- Germany
- Greece
- Hungary
- Ireland
- Italy
- Latvia
- Lithuania
- Luxembourg
- Malta
- Netherlands
- Norway
- Poland
- Portugal
- Romania
- Slovakia
- Slovenia
- Spain
- Sweden
- Switzerland
- UK

The TIGRIS System and TIGRIS System Software are currently marketed for other APTIMA assays in the following countries:

- Australia
- Austria
- Bahamas
- Belgium
- Bermuda
- Bulgaria
- Cambodia
- Canada
- Czech Republic
- Denmark
- Dominican Republic
- Estonia
- Finland
- France
- Germany
- Greece
- Honduras
- Hong Kong
- Hungary
- Iceland
- Ireland
- Israel
- Italy
- Japan
- Kenya
- Latvia
- Libya
- Lithuania
- Luxembourg
- Madagascar
- Malaysia
- Mali
- Malta
- Namibia
- Netherlands
- Norway
- Pakistan
- Philippines
- Poland
- Portugal
- Puerto Rico
- Romania
- Slovakia
- Slovenia
- South Africa
- Spain
- Sweden
- Switzerland
- Turkey
- UK
- US

The APTIMA HPV 16 18/45 Genotype Assay has not been withdrawn from marketing for any reason related to its safety or effectiveness.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Below is a list of the potential adverse effects (e.g., complications) associated with the use of the device. As with any *in vitro* diagnostic test, the potential adverse effects are associated with incorrect test results or result interpretations. Failure of this device to perform as expected or failure to correctly interpret results may lead to incorrect HPV test results and subsequently, improper patient management decisions in cervical cancer screening and treatment. False negative results may lead to delays in the timely diagnosis of cervical cancer and treatment, allowing an undetected condition to worsen and potentially increasing morbidity and mortality. False positive results could lead many women to unnecessarily undergo more frequent screening and potentially invasive procedures such as colposcopy and biopsy.

IX. SUMMARY OF PRECLINICAL STUDIES

A. Laboratory Studies

Clinical Cutoff Determination for the APTIMA HPV 16 18/45 Genotype Assay.
The clinical cutoff for detecting high grade cervical disease (≥CIN2) for the APTIMA HPV 16 18/45 Genotype Assay was verified with specimens from women with APTIMA HPV Assay positive results from the ASC-US and NILM populations in the CLEAR trial. The cutoff was set at 1.0 S/CO for both HPV 16 and HPV 18/45.

Limit of Detection at the Clinical Cutoff
The Limit of Detection (LOD) at the clinical cutoff is a concentration that is positive (above the clinical cutoff) 95% of the time. The LOD of the APTIMA HPV 16 18/45 Genotype Assay was estimated by testing individual or pools of negative clinical ThinPrep liquid cytology specimens spiked with HPV in vitro transcripts or HPV-infected cultured cells (SiHa, HeLa, and MS751; ATCC, Manassas, Virginia) at various concentrations. Thirty replicates of each copy level were tested with each of three reagent lots for a total of 90 replicates. Testing was performed over six days, with three runs performed per day and five replicates of a given genotype tested in each run. The 95% detection limit was calculated from Probit regression analysis of the positivity results for each dilutional panel and is shown in the table below.

Limit of Detection at the Clinical Cutoff

Target	Limit of Detection copies/reaction (95% CI)
HPV 16	57.3 (46.5 - 74.6)
HPV 18	84.8 (66.1 - 115.6)
HPV 45	60.0 (46.6 - 82.3)
SiHa	1.2 (0.9 - 1.7)
HeLa	0.4 (0.3 - 0.5)
MS751	2.6 (1.9 - 4.2)

Assay Precision
APTIMA HPV 16 18/45 Genotype Assay precision was evaluated in two studies using the same 22-member panel. Study 1 was conducted at three external testing sites to determine assay reproducibility. Study 2 was conducted in-house to determine within-laboratory precision. The panel included 14 HPV 16 and/or 18/45-positive members with concentrations at or above the limit of detection of the assay (expected positivity: ≥ 95%), five HPV 16 and/or 18/45- positive members with concentrations below the limit of detection of the assay (expected positivity: >0% to <25%), and three HPV-negative members. HPV 16 and/or 18/45-positive panel members were prepared by spiking HPV-infected cultured cells (SiHa, HeLa, and MS751; ATCC, Manassas, Virginia) into pooled residual ThinPrep liquid cytology specimens or diluting HPV 16, 18, and/or 45 clinical specimens into pooled residual ThinPrep liquid cytology specimens. HPV-negative panel members were prepared with pooled ThinPrep liquid cytology specimens.

In Study 1, two operators at each of the three testing sites (one instrument per site) performed two APTIMA HPV 16 18/45 Genotype Assay worklists per day over three days. Testing was performed using one reagent lot. Each worklist contained three replicates of each of the reproducibility panel members. One hundred eight (108) individual sample tubes were tested for each panel member (3 sites x 1 instrument x 2 operators x 1 lot x 2 worklists per day x 3 days x 3 replicates). In Study 2, testing was conducted in-house over 20 days with a total of 162 reactions tested for each panel member (1 site x 3 instruments x 3 operators x 3 lots x 2 worklists x 3 replicates).

The panel members are described in the tables below, along with a summary of the agreement with expected results for HPV 16 and HPV 18/45 respectively.

APTIMA HPV 16 18/45 Genotype Assay Precision Study 1 and 2: Panel Description and Percent Agreement With HPV 16 Expected Results

Panel Description (copies of cells/reaction)	HPV 16 Expected Result	Percent Agreement (95% CI)	
		Study 1 (3 testing sites)	Study 2 (1 testing site)
SiHa cells (3.0 cells)	Positive	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HeLa cells (0.6 cells)	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
MS751 cells (11.0 cells)	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV 16 clinical sample 1	Positive	100 (107/107) (96.5, 100)	100 (162/162) (97.7, 100)
HPV 18/45 clinical sample 1	Negative	100 (108/108) (96.6, 100)	98.8 (160/162) (95.6, 99.7)
SiHa cells (1.6 cells) & HeLa cells (3.3 cells)	Positive	100 (108/108) (96.6, 100)	98.8 (160/162) (95.6, 99.7)
SiHa cells (1.6 cells) & MS751 cells (42.5 cells)	Positive	100 (108/108) (96.6, 100)	99.4 (161/162) (96.6, 99.9)
SiHa cells (15.7 cells) & HeLa cells (0.3 cells)	Positive	100 (108/108) (96.6, 100)	100 (161/161) (97.7, 100)
SiHa cells (15.7 cells) & MS751 cells (4.3 cells)	Positive	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
SiHa cells (1.6 cells)	Positive	97.2 (105/108) (92.1, 99.1)	98.8 (160/162) (95.6, 99.7)
HeLa cells (0.3 cells)	Negative	100 (108/108) (96.6, 100)	100 (161/161) (97.7, 100)
MS751 cells (4.3 cells)	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV 16 clinical sample 2	Positive	97.2 (104/107) (92.1, 99.0)	94.4 (152/161) (88.7, 97.0)
HPV 18/45 clinical sample 2	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
SiHa cells (0.1 cells)	Negative	85.2 (92/108) (77.3, 90.7)	84.6 (137/162) (78.2, 89.3)
HeLa cells (0.02 cells)	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
MS751 cells (0.04 cells)	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV 16 clinical sample 3	Negative	95.4 (103/108) (89.6, 98.0)	92.6 (150/162) (87.5, 95.7)
HPV 18/45 clinical sample 3	Negative	100 (108/108) (96.6, 100)	99.4 (161/162) (96.6, 99.9)

Panel Description (copies of cells/reaction)	HPV 16 Expected Result	Percent Agreement (95% CI)	
		Study 1 (3 testing sites)	Study 2 (1 testing site)
HPV-negative clinical sample 1	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV-negative clinical sample 2	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV-negative clinical sample 3	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)

CI = Score Confidence Interval
Note: The percent agreement may have been affected by variations in spiking, diluting, and/or aliquoting.

APTIMA HPV 16 18/45 Genotype Assay Precision Study 1 and 2: Panel Description and Percent Agreement With HPV 18/45 Expected Results

Panel Description (copies of cells/reaction)	Percent Agreement (95% CI)		
	HPV 18/45 Expected Result	Study 1 (3 testing sites)	Study 2 (1 testing site)
SiHa cells (3.0 cells)	Negative	100 (108/108) (96.6, 100)	98.8 (160/162) (95.6, 99.7)
HeLa cells (0.6 cells)	Positive	93.5 (101/108) (87.2, 96.8)	98.1 (159/162) (94.7, 99.4)
MS751 cells (11.0 cells)	Positive	92.6 (100/108) (86.1, 96.2)	92.6 (150/162) (87.5, 95.7)
HPV 16 clinical sample 1	Negative	100 (107/107) (96.5, 100)	100 (162/162) (97.7, 100)
HPV 18/45 clinical sample 1	Positive	99.1 (107/108) (94.9, 99.8)	99.4 (161/162) (96.6, 99.9)
SiHa cells (1.6 cells) & HeLa cells (3.3 cells)	Positive	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
SiHa cells (1.6 cells) & MS751 cells (42.5 cells)	Positive	100 (108/108) (96.6, 100)	99.4 (161/162) (96.6, 99.9)
SiHa cells (15.7 cells) & HeLa cells (0.3 cells)	Positive	63.9 (69/108) (54.5, 72.3)	67.7 (109/161) (60.1, 74.4)
SiHa cells (15.7 cells) & MS751 cells (4.3 cells)	Positive	98.1 (106/108) (93.5, 99.5)	92.0 (149/162) (86.8, 95.3)
SiHa cells (1.6 cells)	Negative	100 (108/108) (96.6, 100)	99.4 (161/162) (96.6, 99.9)
HeLa cells (0.3 cells)	Positive	71.3 (77/108) (62.1, 79.0)	92.5 (149/161) (87.4, 95.7)
MS751 cells (4.3 cells)	Positive	86.1 (93/108) (78.3, 91.4)	69.1 (112/162) (61.6, 75.7)
HPV 16 clinical sample 2	Negative	100 (107/107) (96.5, 100)	99.4 (160/161) (96.6, 99.9)
HPV 18/45 clinical sample 2	Positive	88.0 (95/108) (80.5, 92.8)	79.6 (129/162) (72.8, 85.1)
SiHa cells (0.1 cells)	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HeLa cells (0.02 cells)	Negative	92.6 (100/108) (86.1, 96.2)	86.4 (140/162) (80.3, 90.9)
MS751 cells (0.04 cells)	Negative	97.2 (105/108) (92.1, 99.1)	98.1 (159/162) (94.7, 99.4)
HPV 16 clinical sample 3	Negative	100 (108/108) (96.6, 100)	99.4 (161/162) (96.6, 99.9)

Panel Description (copies of cells/reaction)	Percent Agreement (95% CI)		
	HPV 18/45 Expected Result	Study 1 (3 testing sites)	Study 2 (1 testing site)
HPV 18/45 clinical sample 3	Negative	80.6 (87/108) (72.1, 86.9)	81.5 (132/162) (74.8, 86.7)
HPV-negative clinical sample 1	Negative	100 (108/108) (96.6, 100)	99.4 (161/162) (96.6, 99.9)
HPV-negative clinical sample 2	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV-negative clinical sample 3	Negative	100 (108/108) (96.6, 100)	99.4(161/162) (96.6, 99.9)

CI = Score Confidence Interval
Note: The percent agreement may have been affected by variations in spiking, diluting, and/or aliquoting.

The table below presents the HPV 16 and HPV 18/45 analyte S/CO values at the 2.5th, the 50th, and 97.5th percentiles of the S/CO distribution.

APTIMA HPV 16 18/45 Genotype Assay Precision Study 1 and 2: Percentile Distribution of HPV 16 and HPV 18/45 Analyte S/CO Values

Panel Description (copies of cells/reaction)	HPV 16 Analyte S/CO Percentile						HPV 18/45 Analyte S/CO Percentile					
	Study 1 (3 testing sites)			Study 2 (1 testing site)			Study 1 (3 testing sites)			Study 2 (1 testing site)		
	2.5th	50th	97.5th	2.5th	50th	97.5th	2.5th	50th	97.5th	2.5th	50th	97.5th
SiHa cells (3.0 cells)	1.43	3.30	3.89	2.21	3.36	3.86	0.00	0.00	0.25	0.00	0.00	0.00
HeLa cells (0.6 cells)	0.02	0.26	0.49	0.02	0.27	0.46	0.37	3.96	5.33	1.09	3.95	5.17
MS751 cells (11.0 cells)	0.25	0.37	0.64	0.22	0.36	0.57	0.68	3.67	4.51	0.61	2.80	4.29
HPV 16 clinical sample 1	2.70	3.74	4.17	3.53	3.94	4.42	0.00	0.00	0.00	0.00	0.00	0.00
HPV 18/45 clinical sample 1	0.05	0.36	0.71	0.08	0.35	0.71	1.24	4.68	7.25	1.62	4.08	6.20
SiHa cells (1.6 cells) & HeLa cells (3.3 cells)	1.44	3.43	4.34	1.28	3.22	4.35	3.24	4.20	5.01	3.07	4.04	5.04
SiHa cells (1.6 cells) & MS751 cells (42.5 cells)	1.53	3.28	4.14	1.41	3.26	4.18	3.14	3.78	4.37	2.77	3.69	4.23
SiHa cells (15.7 cells) & HeLa cells (0.3 cells)	3.11	3.81	4.47	3.35	4.01	4.75	0.00	1.86	4.08	0.00	1.75	4.11
SiHa cells (15.7 cells) & MS751 cells (4.3 cells)	3.02	3.90	4.54	3.42	4.01	4.64	1.18	3.27	4.34	0.64	2.89	3.95
SiHa cells (1.6 cells)	0.83	3.30	3.91	1.23	3.23	3.90	0.00	0.00	0.01	0.00	0.00	0.13
HeLa cells (0.3 cells)	0.04	0.30	0.49	0.00	0.26	0.47	0.00	2.63	4.81	0.44	3.57	4.95
MS751 cells (4.3 cells)	0.16	0.35	0.59	0.23	0.34	0.51	0.25	2.34	4.48	0.17	1.69	3.75
HPV 16 clinical sample 2	0.89	2.78	3.63	0.82	2.66	3.95	0.00	0.00	0.00	0.00	0.00	0.00

Panel Description (copies of cells/reaction)	HPV 16 Analyte S/CO Percentile						HPV 18/45 Analyte S/CO Percentile					
	Study 1 (3 testing sites)			Study 2 (1 testing site)			Study 1 (3 testing sites)			Study 2 (1 testing site)		
	2.5th	50th	97.5th	2.5th	50th	97.5th	2.5th	50th	97.5th	2.5th	50th	97.5th
HPV 18/45 clinical sample 2	0.24	0.34	0.63	0.23	0.34	0.56	0.44	2.58	4.41	0.27	2.35	4.43
SiHa cells (0.1 cells)	0.28	0.31	2.70	0.27	0.33	2.62	0.00	0.00	0.07	0.00	0.00	0.07
HeLa cells (0.02 cells)	0.25	0.31	0.38	0.18	0.30	0.35	0.00	0.02	2.72	0.00	0.01	2.42
MS751 cells (0.04 cells)	0.25	0.31	0.38	0.27	0.31	0.35	0.00	0.00	1.03	0.00	0.00	0.84
HPV 16 clinical sample 3	0.25	0.31	3.38	0.28	0.32	3.07	0.00	0.00	0.60	0.00	0.00	0.11
HPV 18/45 clinical sample 3	0.26	0.31	0.53	0.19	0.33	0.80	0.00	0.08	4.39	0.00	0.11	4.70
HPV-negative clinical sample 1	0.27	0.31	0.35	0.28	0.31	0.34	0.00	0.00	0.15	0.00	0.00	0.16
HPV-negative clinical sample 2	0.27	0.31	0.35	0.28	0.31	0.34	0.00	0.00	0.07	0.00	0.00	0.09
HPV-negative clinical sample 3	0.26	0.30	0.34	0.27	0.30	0.33	0.00	0.00	0.05	0.00	0.00	0.30

The HPV 16 analyte S/CO variability is shown in the tables below for Study 1 and Study 2 for the panel members with an expected positive result for HPV 16.

APTIMA HPV 16 18/45 Genotype Assay Precision Study 1: HPV 16 Analyte Signal Variability for Panel Members with an Expected Positive Result for HPV 16

Panel Description (cells/reaction)	N	Mean S/CO	Between Sites		Between Operators		Between Worklists		Within Worklists		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
SiHa cells (3.0 cells)	108	3.19	0.00	0.0	0.21	6.7	0.24	7.6	0.42	13.1	0.53	16.6
HPV 16 clinical sample 1	107*	3.72	0.07	1.8	0.05	1.4	0.17	4.5	0.21	5.7	0.28	7.6
SiHa cells (1.6 cells) & HeLa cells (3.3 cells)	108	3.23	0.00	0.0	0.16	4.8	0.24	7.4	0.70	21.7	0.76	23.4
SiHa cells (1.6 cells) & MS751 cells (42.5 cells)	108	3.14	0.14	4.6	0.19	6.2	0.30	9.6	0.56	17.9	0.68	21.7
SiHa cells (15.7 cells) & HeLa cells (0.3 cells)	108	3.79	0.10	2.7	0.00	0.0	0.22	5.8	0.26	7.0	0.36	9.4
SiHa cells (15.7 cells) & MS751 cells (4.3 cells)	108	3.88	0.11	2.9	<0.01	0.2	<0.04	1.0	0.33	8.4	0.35	9.0
SiHa cells (1.6 cells)	108	2.93	0.20	6.7	0.29	9.9	0.28	9.7	0.76	26.1	0.89	30.3
HPV 16 clinical sample 2	107*	2.58	0.24	9.5	0.08	3.2	0.24	9.4	0.77	29.8	0.85	32.8

SD = Standard Deviation, CV = Coefficient of Variation, S/CO = Signal to Cutoff ratio
*Two samples had invalid APTIMA HPV 16 18/45 Genotype Assay results and were not included in the analyses.
Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as zero.

APTIMA HPV 16 18/45 Genotype Assay Precision Study 2: HPV 16 Analyte Signal Variability for Panel Members with an Expected Positive Result for HPV 16

Panel Description (cells/reaction)	N	Mean S/CO	Between Instruments		Between Operators		Between Lots		Between Worklists		Within Worklists		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
SiHa cells (3.0 cells)	162	3.27	0.00	0.0	0.00	0.0	0.00	0.0	0.16	4.8	0.43	13.1	0.46	13.9
HPV 16 clinical sample 1	162	3.95	0.06	1.6	0.09	2.2	0.15	3.8	0.09	2.2	0.24	6.0	0.31	7.9
SiHa cells (1.6 cells) & HeLa cells (3.3 cells)	162	3.08	0.17	5.5	0.15	4.8	0.28	9.0	0.49	16.0	0.59	19.2	0.85	27.5
SiHa cells (1.6 cells) & MS751 cells (42.5 cells)	162	3.08	0.00	0.0	0.00	0.0	0.15	4.9	0.50	16.2	0.59	19.0	0.78	25.4
SiHa cells (15.7 cells) & HeLa cells (0.3 cells)	161*	4.02	0.15	3.7	0.08	2.1	0.18	4.5	0.07	1.8	0.30	7.6	0.40	9.9
SiHa cells (15.7 cells) & MS751 cells (4.3 cells)	162	4.01	0.10	2.5	0.05	1.2	0.13	3.3	0.00	0.0	0.31	7.7	0.35	8.8
SiHa cells (1.6 cells)	162	2.98	0.09	3.0	0.13	4.2	0.30	10.2	0.37	12.3	0.57	19.1	0.76	25.4
HPV 16 clinical sample 2	161*	2.58	0.00	0.0	0.00	0.0	0.29	11.1	0.54	20.9	0.67	25.9	0.91	35.1

SD = Standard Deviation, CV = Coefficient of Variation, S/CO = Signal to Cutoff ratio
*Two samples had invalid APTIMA HPV 16 18/45 Genotype Assay results and were not included in the analyses.
Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as zero.

The HPV 18/45 analyte S/CO variability is shown in the tables below for Study 1 and Study 2 for the panel members with an expected positive result for HPV 18/45.

APTIMA HPV 16 18/45 Genotype Assay Precision Study 1: HPV 18/45 Analyte Signal Variability for Panel Members with an Expected Positive Result for HPV 18/45

Panel Description (cells/reaction)	N	Mean S/CO	Between Sites		Between Operators		Between Worklists		Within Worklists		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
HeLa cells (0.6 cells)	108	3.62	0.00	0.0	0.36	9.9	0.00	0.0	1.30	35.9	1.35	37.2
MS751 cells (11.0 cells)	108	3.30	0.00	0.0	0.39	11.9	0.00	0.0	1.03	31.1	1.10	33.3
HPV 18/45 clinical sample 1	108	4.61	0.00	0.0	0.28	6.1	0.00	0.0	1.35	29.3	1.38	29.9
SiHa cells (1.6 cells) & HeLa cells (3.3 cells)	108	4.19	0.04	1.1	0.15	3.6	0.00	0.0	0.41	9.9	0.44	10.6
SiHa cells (1.6 cells) & MS751 cells (42.5 cells)	108	3.80	0.08	2.0	0.09	2.4	0.14	3.8	0.29	7.8	0.35	9.2
SiHa cells (15.7 cells) & HeLa cells (0.3 cells)	108	1.86	0.00	0.0	0.46	24.8	0.00	0.0	1.32	71.0	1.40	75.3

Panel Description (cells/reaction)	N	Mean S/CO	Between Sites		Between Operators		Between Worklists		Within Worklists		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
SiHa cells (15.7 cells) & MS751 cells (4.3 cells)	108	3.07	0.00	0.0	<0.01	0.0	0.26	8.4	0.76	24.9	0.81	26.3
HeLa cells (0.3 cells)	108	2.40	0.00	0.0	0.45	18.6	0.00	0.0	1.61	67.2	1.67	69.8
MS751 cells (4.3 cells)	108	2.39	0.00	0.0	0.30	12.6	0.41	17.1	1.10	45.9	1.21	50.6
HPV 18/45 clinical sample 2	108	2.61	0.00	0.0	0.23	9.0	0.16	5.9	1.19	45.5	1.22	46.7

SD = Standard Deviation, CV = Coefficient of Variation, S/CO = Signal to Cutoff ratio
Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as zero.

APTIMA HPV 16 18/45 Genotype Assay Precision Study 2: HPV 18/45 Analyte Signal Variability for Panel Members with an Expected Positive Result for HPV 18/45

Panel Description (cells/reaction)	N	Mean S/CO	Between Instruments		Between Operators		Between Lots		Between Worklists		Within Worklists		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
HeLa cells (0.6 cells)	162	3.67	0.15	3.9	0.05	1.3	0.46	12.6	0.67	18.3	0.74	20.1	1.11	30.2
MS751 cells (11.0 cells)	162	2.69	0.05	1.8	0.00	0.0	0.00	0.0	0.34	12.8	1.02	38.0	1.08	40.1
HPV 18/45 clinical sample 1	162	4.01	0.17	4.2	0.00	0.0	0.20	4.9	0.74	18.4	0.97	24.1	1.24	31.0
SiHa cells (1.6 cells) & HeLa cells (3.3 cells)	162	4.06	0.26	6.4	0.07	1.7	0.21	5.0	0.14	3.3	0.43	10.6	0.56	13.9
SiHa cells (1.6 cells) & MS751 cells (42.5 cells)	162	3.63	0.20	5.5	0.00	0.0	0.09	2.5	0.10	2.9	0.38	10.4	0.45	12.3
SiHa cells (15.7 cells) & HeLa cells (0.3 cells)	161*	1.71	0.00	0.0	0.28	16.1	0.34	19.6	0.85	49.9	0.79	46.4	1.25	72.7
SiHa cells (15.7 cells) & MS751 cells (4.3 cells)	162	2.62	0.31	11.9	0.00	0.0	0.17	6.6	0.24	9.1	0.89	33.8	0.98	37.6
HeLa cells (0.3 cells)	161*	3.25	0.31	9.5	0.17	5.3	0.31	9.4	0.75	23.1	0.89	27.2	1.25	38.5
MS751 cells (4.3 cells)	162	1.84	0.00	0.0	0.00	0.0	0.21	11.5	0.44	24.1	1.02	55.4	1.13	61.5
HPV 18/45 clinical sample 2	162	2.38	0.44	18.6	0.00	0.0	0.00	0.0	0.95	39.8	0.90	37.8	1.38	58.0

SD = Standard Deviation, CV = Coefficient of Variation, S/CO = Signal to Cutoff ratio
*Two samples had invalid APTIMA HPV 16 18/45 Genotype Assay results and were not included in the analyses.
Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as zero.

A third study was also conducted to determine within-laboratory precision by testing a nine-member panel comprised of *in vitro* transcript (IVT) spiked into a matrix of PreservCyt solution diluted 1:2.9 in STM. One panel member was HPV negative. Four panel members were low positive, with IVT (HPV 16, HPV 18, HPV 45, HPV 18 and HPV 45) spiked at the limit of detection of the assay (expected positivity: ≥ 95%). Four panel members were moderate positive, with IVT (HPV 16, HPV 18, HPV 45, HPV 18 and HPV 45) spiked above the limit of detection of the assay (~3 x the detection limit; expected positivity: 100%). Testing was conducted in-house by three operators using two reagent lots, three instruments, over nine days, testing two runs per day in which the panel was tested in triplicate. The panel members are described in the table below, along with a summary of the agreement with expected results (HPV 16 and HPV 18/45).

APTIMA HPV 16 18/45 Genotype Assay Precision Study 3: Agreement with Expected Results

Description (copies/reaction)	Expected Result	Valid N	HPV 16 Agreement			HPV 18/45 Agreement		
			N	%	95% CI	N	%	95% CI
HPV 16 IVT (60 copies)	16 Pos, 18/45 Neg	108	108	100	96.6, 100	108	100	96.6, 100
HPV 18 IVT (85 copies)	16 Neg, 18/45 Pos	108	108	100	96.6, 100	108	100	96.6, 100
HPV 45 IVT (60 copies)	16 Neg, 18/45 Pos	108	108	100	96.6, 100	108	100	96.6, 100
HPV 18 IVT (85 copies) & HPV 45 IVT (60 copies)	16 Neg, 18/45 Pos	108	107	99.1	94.9, 99.8	108	100	96.6, 100
HPV Negative (0 copies)	16 Neg, 18/45 Neg	108	108	100	96.6, 100	108	100	96.6, 100
HPV 16 IVT (180 copies)	16 Pos, 18.45 Neg	108	108	100	96.6, 100	108	100	96.6, 100
HPV 18 IVT (260 copies)	16 Neg, 18/45 Pos	108	108	100	96.6, 100	108	100	96.6, 100
HPV 45 IVT (180 copies)	16 Neg, 18/45 Pos	108	108	100	96.6, 100	108	100	96.6, 100
HPV 18 IVT (260 copies) & HPV 45 IVT (180 copies)	16 Neg, 18/45 Pos	108	108	100	96.6, 100	108	100	96.6, 100

IVT = in vitro transcript; Neg = Negative; Pos = Positive

The S/CO values for Study 3 at the 2.5th, 50th, and 97.5th percentiles of the signal distribution are shown in the table below.

APTIMA HPV 16 18/45 Genotype Assay Precision Study 3: Percentile Distribution of HPV 16 and HPV 18/45 Analyte S/CO Values

Description (cells/reaction)	HPV 16 S/CO				HPV 18/45 S/CO			
	Mean	2.5th	50th	97.5th	Mean	2.5th	50th	97.5th
HPV 16 IVT (60 copies)	3.71	2.81	3.76	4.33	0.02	0.00	0.00	0.40
HPV 18 IVT (85 copies)	0.30	0.02	0.29	0.57	5.26	4.64	5.23	6.00
HPV 45 IVT (60 copies)	0.36	0.10	0.35	0.66	4.63	3.81	4.57	5.55
HPV 18 IVT (85 copies) & HPV 45 IVT (60 copies)	0.40	0.05	0.40	0.84	7.85	6.90	7.82	8.92
HPV Negative (0 copies)	0.32	0.26	0.31	0.36	0.00	0.00	0.00	0.02
HPV 16 IVT (180 copies)	3.94	13.38	3.94	4.52	0.00	0.00	0.00	0.00
HPV 18 IVT (260 copies)	0.29	0.00	0.30	0.60	5.38	4.76	5.38	6.00
HPV 45 IVT (180 copies)	0.37	0.02	0.36	0.68	4.72	3.96	4.70	5.52
HPV 18 IVT (260 copies) & HPV 45 IVT (180 copies)	0.41	0.04	0.42	0.86	7.85	6.94	7.82	8.66

IVT = in vitro transcript

The HPV 16 analyte S/ CO variability for Study 3 is shown in the table below for the panel members with an expected positive result for HPV 16.

APTIMA HPV 18/45 Genotype Assay Precision Study 3: HPV 16 Analyte Signal Variability for Panel Members with an Expected Positive Result for HPV 16

Description (cells/reaction)	N	Mean S/CO	Inter-instrument		Inter-operator		Inter-lot		Inter-Run		Intra-Run		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
HPV 16 IVT (60 copies)	108	3.71	0.07	1.9	0.10	2.6	0.06	1.5	0.09	2.4	0.33	8.9	0.37	10.0
HPV 16 IVT (180 copies)	108	3.94	0.00	0.0	0.07	1.8	0.18	4.6	0.15	3.7	0.18	4.4	0.30	7.6

IVT = in vitro transcript

Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as zero

The HPV 18/45 analyte S/CO variability for Study 3 is shown in the table below for the panel members with an expected positive result for HPV 18/45..

APTIMA HPV 16 18/45 Genotype Assay Precision Study 3: HPV 18/45 Analyte Signal Variability for Panel Members with an Expected Positive Result for HPV 18/45

Description (cells/reaction)	N	Mean S/CO	Inter-instrument		Inter-operator		Inter-lot		Inter-Run		Intra-Run		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
HPV 18 IVT (85 copies)	108	5.26	0.00	0.0	0.00	0.0	0.03	0.6	0.13	2.5	0.32	6.0	0.35	6.6
HPV 45 IVT (60 copies)	108	4.63	0.11	2.3	0.04	0.9	0.46	10.0	0.00	0.0	0.30	6.4	0.56	12.1
HPV 18 IVT (85 copies) & HPV 45 IVT (60 copies)	108	7.85	0.00	0.0	0.13	1.7	0.32	4.1	0.24	3.1	0.41	5.2	0.59	7.5
HPV 18 IVT (260 copies)	108	5.38	0.00	0.0	0.00	0.0	0.08	1.4	0.07	1.3	0.28	5.3	0.30	5.6
HPV 45 IVT (180 copies)	108	4.72	0.13	2.7	0.06	1.3	0.51	10.7	0.08	1.6	0.16	3.4	0.56	11.9
HPV 18 IVT (260 copies) & HPV 45 IVT (180 copies)	108	7.85	0.00	0.0	0.05	0.6	0.27	3.4	0.18	2.2	0.34	4.3	0.47	6.0

IVT = in vitro transcript

Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as zero.

Cross-Reactivity

The analytical specificity of the APTIMA HPV 16 18/45 Genotype Assay was evaluated with pools of residual ThinPrep liquid cytology specimens diluted 1:2.9 into STM (comparable to specimen transferred to an APTIMA Transfer tube) and spiked with cultured bacteria, yeast, or fungi; cultured virus; or non-targeted HPV *in vitro* transcripts. The organisms and test concentrations for which no cross reactivity was observed are identified in the table below. The study criteria for assessing the effect of the presence of microorganism on the specificity of the assay were based on positivity.

Analytical Specificity Panel: Organisms and Concentration with No Cross-Reactivity

Organism	Test Concentration with No Cross-Reactivity	Organism	Test Concentration with No Cross-Reactivity
Bacteria			
Acinetobacter lwoffii	$1x10^6$ CFU/mL	*Lactobacillus acidophilus*	$1x10^6$ CFU/mL
Actinomyces israelii	$1x10^6$ CFU/mL	*Lactobacillus crispatus*	$1x10^6$ CFU/mL
Alcaligenes faecalis	$1x10^6$ CFU/mL	*Listeria monocytogenes*	$1x10^6$ CFU/mL
Atopobium vaginae	$1x10^6$ CFU/mL	*Mobiluncus curtisii*	$1x10^6$ CFU/mL
Bacteroides fragilis	$1x10^6$ CFU/mL	*Mycoplasma genitalium**	$2.5x10^6$ copies/mL
Bifidobacterium adolescentis	$1x10^6$ CFU/mL	*Mycoplasma hominis*	$1x10^6$ CFU/mL
Campylobacter jejuni	$1x10^6$ CFU/mL	*Neisseria gonorrhoeae*	$1x10^6$ CFU/mL
Chlamydia trachomatis	$1x10^5$ IFU/mL	*Peptostreptococcus magnus*	$1x10^6$ CFU/mL
Clostridium difficile	$1x10^6$ CFU/mL	*Prevotaella bivia*	$1x10^6$ CFU/mL
Corynebacterium genitalium	$1x10^6$ CFU/mL	*Propionibacterium acnes*	$1x10^6$ CFU/mL
Cryptococcus neoformans	$1x10^6$ CFU/mL	*Proteus vulgaris*	$1x10^6$ CFU/mL
Enterobacter cloacae	$1x10^6$ CFU/mL	*Pseudomonas aeruginosa*	$1x10^6$ CFU/mL
Enterococcus faecalis	$1x10^6$ CFU/mL	*Staphylococcus aureus*	$1x10^6$ CFU/mL
Escherichia coli	$1x10^6$ CFU/mL	*Staphylococcus epidermidis*	$1x10^6$ CFU/mL
Fusobacterium nucleatum	$1x10^6$ CFU/mL	*Streptococcus agalactiae*	$1x10^6$ CFU/mL
Gardnerella vaginalis	$1x10^6$ CFU/mL	*Streptococcus pyogenes*	$1x10^6$ CFU/mL
Haemophilus ducreyi	$1x10^6$ CFU/mL	*Ureaplasma urealyticum*	$1x10^6$ CFU/mL
Klebsiella pneumoniae	$1x10^6$ CFU/mL		
Yeast/protozoa			
Candida albicans	$1x10^6$ CFU/mL	*Trichomonas vaginalis***	$1x10^5$ cells/mL
Viruses			
Adenovirus	$5.25x10^7$ PFU/mL	HIV-1	$2.5x10^6$ copies/mL
Cytomegalovirus	$1.58x10^6$ $TCID_{50}$/mL	Herpes simplex virus 1	$3.39x10^6$ $TCID_{50}$/mL
Epstein-Barr virus	$1.59x10^5$ TD_{50}/mL	Herpes simplex virus 2	$2.29x10^6$ $TCID_{50}$/mL

Non-targeted High-risk HPV genotypes*			
HPV 31	$2.5x10^6$ copies/mL	HPV 56	$2.5x10^6$ copies/mL
HPV 33	$2.5x10^6$ copies/mL	HPV 58	$2.5x10^6$ copies/mL
HPV 35	$2.5x10^6$ copies/mL	HPV 59	$2.5x10^6$ copies/mL
HPV 39	$2.5x10^6$ copies/mL	HPV 66	$2.5x10^6$ copies/mL
HPV 51	$2.5x10^6$ copies/mL	HPV 68	$2.5x10^6$ copies/mL
HPV 52	$2.5x10^6$ copies/mL		
Non-targeted other HPV genotypes*			
HPV 6	$2.5x10^6$ copies/mL	HPV 53	$2.5x10^6$ copies/mL
HPV 11	$2.5x10^6$ copies/mL	HPV 67	$2.5x10^6$ copies/mL
HPV 26	$2.5x10^6$ copies/mL	HPV 69	$2.5x10^6$ copies/mL
HPV 30	$2.5x10^6$ copies/mL	HPV 70	$2.5x10^6$ copies/mL
HPV 34	$2.5x10^6$ copies/mL	HPV 73	$2.5x10^6$ copies/mL
HPV 42	$2.5x10^6$ copies/mL	HPV 82	$2.5x10^6$ copies/mL
HPV 43	$2.5x10^6$ copies/mL	HPV 85	$2.5x10^6$ copies/mL
HPV 44	$2.5x10^6$ copies/mL		

CFU = Colony Forming Units, PFU = Plaque Forming Units, TD50 = Transformation Dose 50, TCID50 = Tissue Culture Infective Dose 50

**In vitro* transcript tested.

***Although no cross-reactivity was observed for Trichomonas vaginalis, interference was observed (see below).

The analytical sensitivity of the APTIMA HPV 16 18/45 Genotype Assay in the presence of microorganisms was evaluated with the same panel described in the table above, which was also spiked with a low concentration of HPV infected SiHa cells (1.6 cell per reaction) and HPV infected HeLa cells (0.3 cells/reaction). The study criteria for assessing the effect of the presence of microorganism on the sensitivity of the assay were based on positivity. The presence of the microorganisms did not interfere with the APTIMA HPV 16 18/45 Genotype Assay with the exception of *Trichomonas vaginalis* (TV). Interference was observed with TV when present at concentrations greater than 3×10^4 cells/mL.

Interference

The substances described in the table below were individually spiked into pooled ThinPrep liquid cytology specimens diluted 1:2.9 in STM at the concentrations specified in the table. All substances were tested with the APTIMA HPV 16 18/45 Genotype Assay in the presence and absence of HPV infected cultured cells (SiHa, 1.6 cells/ reaction and HeLa, 0.3 cells/reaction). Interference was observed with the following when present at concentrations greater than those specified: vaginal lubricants (containing Polyquaternium 15) at 1% w/v, anti-fungal cream (containing tioconazole) at 0.03% w/v, mucus at 0.3% w/v, vaginal hormones (containing progesterone) at 1% w/v.

Substances Tested for Possible Interference with the APTIMA HPV 16 18/45 Genotype Assay

Product Category	Product Brand or Type	Highest concentration tested that did not interfere with the assay*
Vaginal Lubricant	KY natural feeling liquid	10% v/v
	Up & up (Target brand) personal lubricant liquid	
	Astroglide	1% w/v
Spermicide/Contraceptive Jelly	Vaginal Contraceptive Foam (VCF)	10% w/v
	Options Conceptrol Vaginal Contraceptive Gel	
Anti-fungal cream	Up & up (Target brand) miconazole 3	10% w/v
	Monistat 3 Combination Pack	
	Up & up (Target brand) Tioconazole 1	0.03% w/v
Douche	Summer's Eve Douche	10% v/v
	Up & up (Target brand) feminine douche	
Feminine Spray	Summer's Eve Feminine Deodorant Spray	10% w/v
	FDS Feminine Deodorant Spray	
Mucus	Porcine mucin	0.3% w/v
Vaginal Hormones	Estrace Vaginal Cream (estrogen)	10% w/v
	Crinone Cream (progesterone)	1% w/v
Whole Blood**	whole blood	5% v/v
Leukocytes	leukocytes	$1x10^7$ cells/mL
Glacial Acetic Acid Wash Solution^	Glacial Acetic Acid + CytoLyt Solution	2.6% v/v

*concentration in the test sample; ThinPrep liquid cytology specimen diluted 1:2.9 into STM (comparable to specimen transferred to an APTIMA Transfer tube)
**whole blood interfered with the assay when present at a 10% v/v test concentration
^glacial acetic acid wash solution prepared by mixing 1 part glacial acetic acid and 9 part Cytolyt solution as denoted in the ThinPrep 2000 System Operator's Manual.

Reagent Stability

Expiration dating for this device has been established and approved at nine months for the APTIMA HPV 16 18/45 Genotype Assay when stored at 2 - 8°C, with the exception of the subset of reagents in the APTIMA HPV 16 18/45 Genotype Room Temperature Box, which should be stored at 15 - 30°C.

Sample Handling and Collection

Cervical specimens should be collected in PreservCyt Solution, the ThinPrep Pap Test preservation system, using a broom-type device (e.g. Rovers Cervex Brush, Wallach Papette), or Endocervical Brush/Spatula.

Specimen stability studies demonstrated that for the APTIMA HPV 16 18/45 Genotype Assay cervical specimens should be transported and stored at 2°C to 30°C, with no more than 30 days at temperatures above 8°C. PreservCyt specimens should be transferred to an APTIMA Specimen Transfer tube within 105 days of collection. PreservCyt Solution specimens transferred to an APTIMA Specimen Transfer tube may be stored at 2°C to 30°C for up to 60 days. If longer storage is needed, the PreservCyt Solution specimen or the PreservCyt Solution specimen diluted into the Specimen Transfer tube may be stored at -20°C for up to 24 months.

TIGRIS DTS System Carryover

Two studies were conducted to determine the rate of false positive results observed with the APTIMA HPV 16 18/45 Genotype Assay using the TIGRIS DTS System when samples containing high titer HPV were interspersed throughout specimen processing racks containing HPV-negative samples. High titer positive samples for this study were created by spiking 10,000 cells/mL of HPV 16 positive SiHa and HPV 18 positive HeLa cells into a pool of HPV-negative residual ThinPrep liquid Pap specimens. In Study 1, three TIGRIS DTS instruments were tested (5 runs per instrument). When 1,026 negative samples without any positive samples were tested the false positive rate was 0.10% (1/1026) with 95% CI: 0.02% to 0.55%; the false positive rate for negative samples that proceeded or followed a high titer positive sample was 0.26% (1/390) with 95% CI: 0.05% to 1.43%. The observed increase in false positive rates was not statistically significant (difference in false positive rates was 0.16% with 95% CI:-0.34% to 1.34%). In Study 2, two TIGRIS DTS instruments were tested (4 runs per instrument). When 470 negative samples without any positive samples were tested the false positive rate was 0.21% (1/470) with 95% CI: 0.04% to 1.20%; the false positive rate for negative samples that proceeded or followed a positive sample was 0.35% (1/282) with 95% CI: 0.06% to 1.98%; the observed increase in false positive rates was not statistically significant (difference was 0.14% with 95% CI: -0.88% to 1.78%).

The observed rate of sample to sample cross-contamination of the APTIMA HPV 16 18/45 Genotype Assay on the TIGRIS DTS System was approximately 0.4%.

ThinPrep Carryover Study

A study was conducted to determine the false positive rate observed with the APTIMA HPV 16 18/45 Genotype Assay when pooled residual PreservCyt liquid Pap specimens containing a high concentration of spiked HPV-positive cells (10,000 cells/mL of both SiHa and HeLa), were alternately processed with pooled clinical HPV-negative specimens on the ThinPrep 2000 Processor (T2000). After the T2000 processing was completed, 1 mL of each sample was transferred to a tube containing STM to create the post-processed samples. A single replicate of each sample was tested (two TIGRIS DTS instruments, three runs per instrument).

In the study, 160 negative samples were tested without any positive samples and the false positive rate was 1.25% (2/160) with 95% CI: 0.34% to 4.44%; the false positive rate for the negative samples followed by a positive sample was 0.63% (1/160) with 95% CI: 0.11% to 3.45%. The difference in false positive rates was not statistically significant (difference was -0.62% with 95% CI: -3.86% to 2.35%). The observed false positive rate for specimens tested with the APTIMA HPV 16 18/45 Genotype Assay following processing on the T2000 was 0.6% when the cleaning procedure described in the APTIMA Specimen Transfer Kit package insert was followed. Users should follow the cleaning instructions provided via the APTIMA Specimen Transfer kit for decontamination between specimens to minimize carryover risk.

B. <u>Animal Studies</u>

Not applicable

C. **Additional Studies**
Not applicable

X. SUMMARY OF PRIMARY CLINICAL STUDIES

A. Study Design

The APTIMA HPV 16 18/45 Genotype Assay was evaluated using referral cytology specimens collected during the APTIMA HPV Assay Clinical Study, also known as the CLEAR trial (P100042[1]). Patients were enrolled between March 2008 and December 2009. This was a prospective cohort, multi-center trial consisting of two sub-studies to support the two distinct indications for use of the assay, the ASC-US Study and the NILM (negative for intraepithelial lesions or malignancy) Study. The database for this PMA included 12,896 patients age 21 and older. There were three APTIMA HPV 16 18/45 Genotype testing sites.

1. Clinical Inclusion and Exclusion Criteria

Enrollment in the APTIMA HPV Assay Clinical Study was limited to patients who attended a participating clinic and underwent a routine cytology test. In addition, the subject must have been able to comprehend and sign an IRB-approved Informed Consent Form and other applicable study enrollment documents. To be included in the ASC-US Study, the subject's referral cytology specimen must have had ASC-US results. To be included in the NILM Study, the subject must have been ≥30 years of age and the subject's referral cytology specimen must have had NILM cytology test results. Note that all women under age 21 that were enrolled in the ASC-US study have been removed from the dataset (n=99), including patient demographics and subject accountability. The approved indication does not cover this age range since HPV testing is not recommended in women under the age of 21[2].

Patients were not permitted to enroll in the either study if the subject, clinician, or medical record reported any of the following:

- History of cervical disease (cancer or precancerous condition) in the previous 12 months
- History of an abnormal cytology test result in the previous 12 months
- Under 18 years of age, without the documented consent of her parent or legal guardian
- Subject is known to be pregnant at enrollment
- History of illness that the investigator considers could interfere with or affect the conduct, results, and/or completion of the clinical trial
- History of illness that the investigator/physician considers to create an unacceptable risk to the subject if enrolled
- History of HPV vaccination prior to enrollment.

Subjects were defined as evaluable for the APTIMA HPV 16 18/45 Genotype Assay study if they were evaluable for the Aptima HPV Assay Clinical Study. ASC-US Study subjects were evaluable if they had a valid Aptima HPV Assay result, attended the colposcopy visit, and were not withdrawn due to not meeting study eligibility criteria, incomplete study documentation, or compliance issues. Adjunct Study subjects were evaluable if they had a valid Aptima HPV Assay result and were not withdrawn due to not meeting eligibility criteria, incomplete study documentation, or compliance issues. Previously frozen samples from evaluable ASC-US Study subjects' referral Pap specimens (in the Aptima Specimen Transfer tube) were tested with the APTIMA HPV 16 18/45 Genotype Assay. Previously frozen samples from evaluable NILM Study subjects' referral Pap specimens (in the Aptima Specimen Transfer tube) were tested with the

APTIMA HPV 16 18/45 Genotype Assay if the following criteria were met: the referral Pap specimen had a valid Aptima HPV Assay result, and the subject attended colposcopy, or the subject did not attend colposcopy but the referral Pap specimen had an Aptima HPV Assay positive result.

2. Follow-up Schedule

Patients were scheduled to return for follow-up examinations as described below.

APTIMA HPV 16 18/45 Genotype Assay Clinical Trial Study Design

The APTIMA HPV 16 18/45 Genotype Assay was evaluated using referral cytology specimens collected from consenting women during the prospective, multicenter US clinical study known as the CLEAR trial. The CLEAR trial was conducted to determine the clinical performance of the APTIMA HPV Assay for detection of cervical intraepithelial neoplasia grade 2 or more severe cervical disease (≥CIN2). Women were enrolled into either the ASC-US Study or the NILM Study based on their referral ThinPrep liquid based cytology results from routine cervical cancer screening. The ASC-US Study population included women 21 years and older with ASC-US cytology results and the NILM Study population included women 30 years of age and older with NILM cytology results.

Women from 18 clinical sites, primarily obstetrics/gynecology clinics, which covered a wide geographic distribution and a diverse population, were analyzed. During the CLEAR trial, residual referral cytology specimens were tested with both the APTIMA HPV Assay and an FDA-approved HPV DNA test. For the APTIMA HPV 16 18/45 Genotype Assay clinical trial, samples from the residual referral cytology specimens were tested with the APTIMA HPV 16 18/45 Genotype Assay.

All women in the ASC-US Study were referred to colposcopy, regardless of their APTIMA HPV Assay and FDA-approved HPV DNA test results. An endocervical curettage (ECC) biopsy and cervical punch biopsies (one biopsy from each of the four quadrants) were obtained. If a lesion was visible, a punch biopsy was obtained (directed method; one biopsy per lesion) and quadrants without a visible lesion were biopsied at the squamocolumnar junction (random method).

In the NILM Study, women positive with the APTIMA HPV Assay and/or the FDA-approved HPV DNA test, as well as randomly selected women who were negative with both assays, were referred to colposcopy for the baseline evaluation. An ECC biopsy was obtained from each woman who attended colposcopy. Punch biopsies were obtained from visible lesions only (direct method; one biopsy per lesion). Follow-up of women in the NILM Study who did not have ≥CIN2 at baseline is ongoing for three years with annual cytology visits. Women with ASC-US or more severe cytology results during the follow-up period are referred to colposcopy using the same biopsy procedure performed for the baseline evaluation.

For both the ASC-US and NILM studies, disease status was determined from a consensus histology review panel, which was based on agreement of at least two expert pathologists. The expert pathologists were masked to the women's HPV and cytology status, as well as each other's histology diagnoses. Investigators, clinicians, and women were masked to the APTIMA HPV Assay and FDA-approved HPV DNA test results until after completion of the colposcopy visit, to avoid bias.

To validate the intended use of the APTIMA HPV 16 18/45 Genotype Assay as a reflex test for an APTIMA HPV Assay positive specimen, residual referral cytology specimens from all evaluable women in the ASC-US Study and the NILM Study with an APTIMA HPV Assay positive result

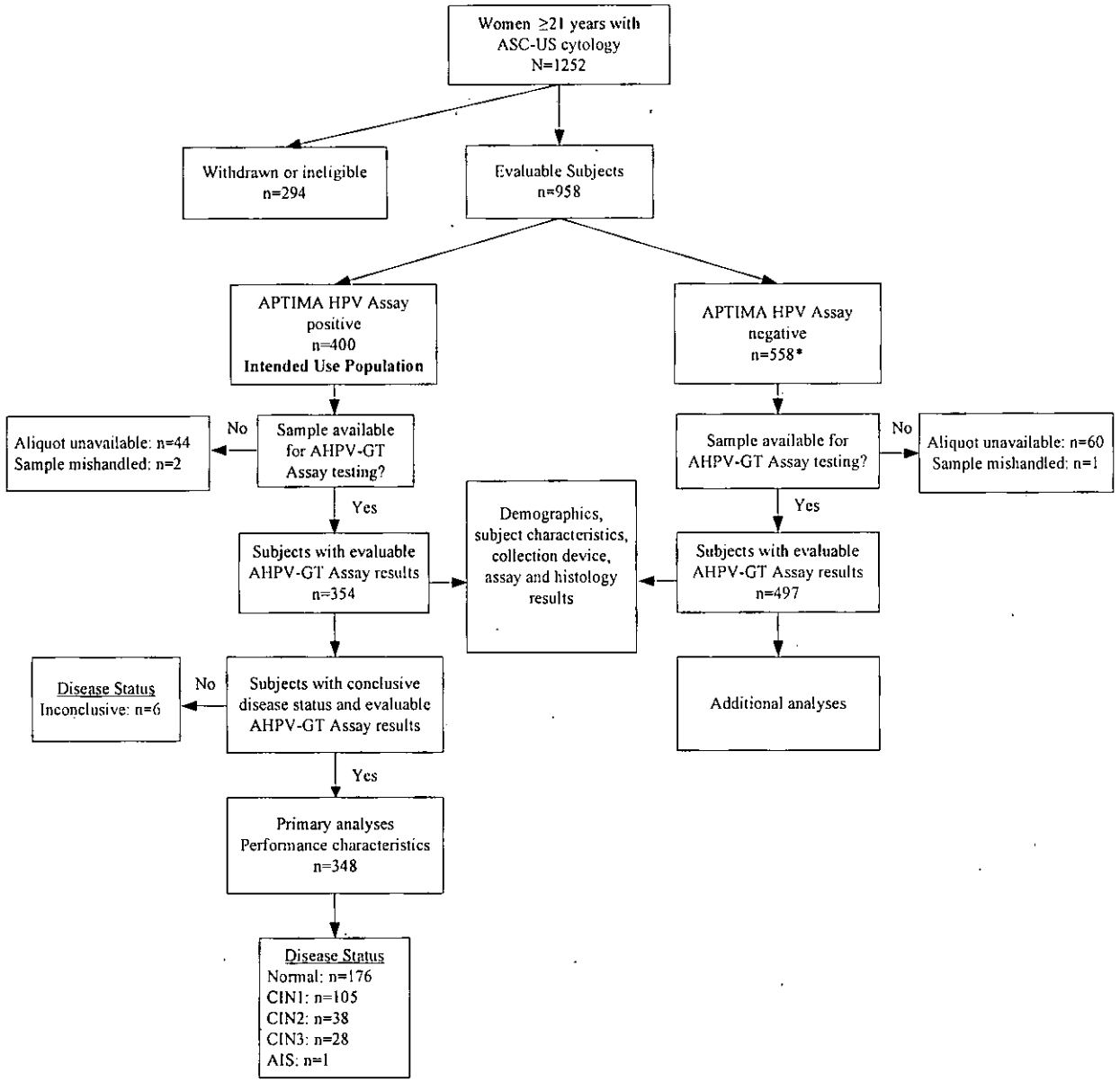
were eligible for testing with the APTIMA HPV 16 18/45 Genotype Assay. Clinical performance of the APTIMA HPV 16 18/45 Genotype Assay for detection of ≥CIN2 and cervical intraepithelial neoplasia grade 3 or more severe cervical disease (≥CIN3) was evaluated.

B. Accountability of PMA Cohort

Accountability in ASC-US (≥ 21 years) Population

The APTIMA HPV 16 18/45 Genotype Assay was evaluated using referral cytology specimens collected during the APTIMA HPV Assay Clinical Study, also known as the CLEAR trial (P100042). Subject enrollment for the ASC-US (≥ 21 years) population began on March 19, 2008, and was completed on December 23, 2009. Colposcopy visits were completed on December 23, 2009. APTIMA HPV 16 18/45 Genotype Assay testing began on June 9, 2011, and was completed on August 19, 2011.

There were 958 evaluable women, 21 years or older, who met the selection criteria and were enrolled into the ASC-US Study. These evaluable women had a valid APTIMA HPV Assay result and attended the colposcopy visit. Of these, 400 subjects were positive with the APTIMA HPV Assay and eligible for testing with the APTIMA HPV 16 18/45 Assay. The ASC-US study enrollment and sample accountability are described below.

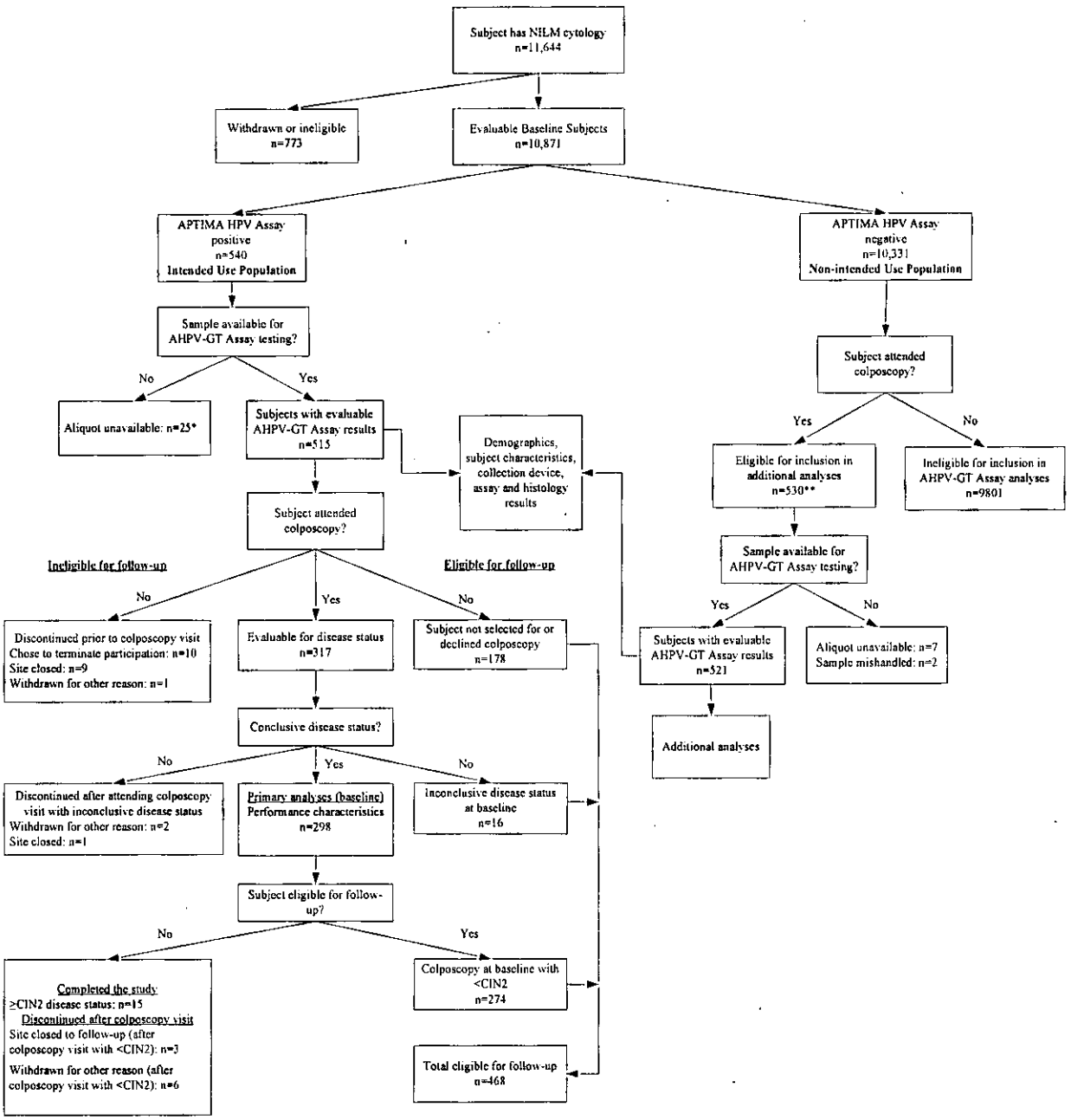


*Includes 545 subjects with conclusive disease status and 13 subjects with inconclusive disease status.

Accountability in NILM (≥ 30 years) population

The APTIMA HPV 16 18/45 Genotype Assay was evaluated using referral cytology specimens collected during the APTIMA HPV Assay Clinical Study, also known as the CLEAR trial (P100042). Subject enrollment for the NILM (≥ 30 years) population began on March 31, 2008, and was completed on December 8, 2009. Baseline colposcopy visits were completed on February 2, 2010. APTIMA HPV 16 18/45 Genotype Assay testing began on June 9, 2011, and was completed on August 19, 2011. Follow-up is currently ongoing and is estimated to be completed by April 2013.

There were 10,871 evaluable women 30 years of age and older with NILM cytology results and APTIMA HPV Assay results. Of these subjects, 540 were positive with the APTIMA HPV Assay and eligible for testing with the APTIMA HPV 16 18/45 Assay. The NILM Study enrollment and sample accountability are described below.



Note: all subjects from the APTIMA HPV Assay NILM Study who had a positive APTIMA HPV Assay results or attended the colposcopy visit were eligible for this study. Only subjects with a positive APTIMA HPV Assay result are part of the intended use population.

*Includes 18 subjects who attended the colposcopy visit and 7 subjects who did not.

**Includes 505 subjects with conclusive disease status and 25 subjects with inconclusive disease status.

C. Study Population Demographics and Baseline Parameters

The demographics of the study populations are typical for a prospective study performed in the US.

Study Demographics

ASC-US Subject Demographics		
Age (years) at consent		
	CLEAR Trial AHPV (N=958)	AHPV+ tested with AHPV-GT (N=354)
Mean	33.8	29.5
SD	10.3	8.0
Median	31.0	27.0
Min	21	21
Max	85	60
Race		
White	587	209
Black or African American	229	103
Asian	29	6
American Indian or Alaska Native	34	14
Native Hawaiian or Pacific Islander	1	0
Unknown / Refused	106	34
Ethnicity		
Hispanic or Latino	195	56
Not Hispanic or Latino	712	284
Unknown / Refused	51	14

NILM Subject Demographics		
Age (years) at consent		
	CLEAR Trial AHPV (N=10871)	AHPV+ tested with AHPV-GT (N=515)
Mean	44.1	41.2
SD	10.1	9.9
Median	43.0	38.0
Min	30	30
Max	89	83
Race		
White	6937	338
Black or African American	1390	63
Asian	658	23
American Indian or Alaska Native	152	12
Native Hawaiian or Pacific Islander	66	3
Unknown / Refused	1808	83
Ethnicity		
Hispanic or Latino	3421	165
Not Hispanic or Latino	6881	327
Unknown / Refused	569	23

AHPV-GT = APTIMA HPV 16 18/45 Genotype Assay, AHPV = APTIMA HPV Assay

D. Safety and Effectiveness Results

ASC-US ≥ 21 Years Population: APTIMA HPV 16 18/45 Genotype Assay Clinical Performance

In total, there were 400 evaluable women 21 years of age and older with ASC-US cytology results and APTIMA HPV Assay positive results whose referral cytology samples were eligible for testing with the APTIMA HPV 16 18/45 Genotype Assay. Of these, 46 women did not have sufficient referral cytology sample volume available for testing in this study and six had undetermined disease diagnoses; after a missing values analysis, they were not included in the performance calculations. The 348 evaluable women with conclusive disease status had valid APTIMA HPV 16 18/45 Genotype Assay results based on reflex testing from an APTIMA HPV Assay positive result. Sixty-seven (67) women had ≥CIN2 and 29 had ≥CIN3.

Of the 348 evaluable women with APTIMA HPV Assay positive results, 117 women had APTIMA HPV 16 18/45 Genotype Assay positive results indicating the presence of HPV 16 and/or HPV 18/45; 231 had negative results, indicating the presence of one or more of the other 11 high-risk HPV types as detected by the APTIMA HPV Assay (i.e., HPV types 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and 68). An additional 545 evaluable women 21 years of age and older with ASC-US cytology results had APTIMA HPV Assay negative results during the CLEAR trial. An APTIMA HPV Assay negative result indicates that none of the 14 high-risk HPV types are present, and were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis. Prevalence of ≥CIN2 and ≥CIN3 in evaluable women with ASC-US cytology results was 8.8% and 3.7% respectively. The results of the APTIMA HPV 16 18/45 Genotype Assay by APTIMA HPV Assay result and consensus histology review panel diagnosis are presented in the table below.

ASC-US ≥ 21 Years Population: Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay by Consensus Histology Review Panel Diagnosis

APTIMA HPV Assay Result	AHPV-GT Assay Result*	Interpretation	Consensus Histology Review Panel Diagnosis						
			Undetermined**	Normal	CIN1	CIN2	CIN3	Cancer	Total
Positive	HPV 16 Neg, HPV 18/45 Neg	Other HR HPV Pos	2	125	73	23	10	0	233
	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos	1	27	18	11	14	0	71
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos	3	23	14	3	3	1	47
	HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	0	1	0	1	1	0	3
Total			6	176	105	38	28	1	354
Negative	HPV 16/18/45 Neg***	HR HPV Neg	13	458	75	8	4	0	558
Total			19	634	180	46	32	1****	912

AHPV-GT = APTIMA HPV 16 18/45 Genotype Assay, CIN1 = Cervical Intraepithelial Neoplasia Grade 1, HR = High-risk, Neg = Negative, Pos = Positive

*All samples had final results (upon final testing or after resolution of initial invalids per procedure).

**19 women attended the colposcopy visit but a diagnosis could not be determined for the following reasons: < 5 biopsy specimens obtained all with histology results of normal/CIN1 (n=15), no biopsies collected (n=3), and biopsy slides lost (n=1).

***Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

****One woman had adenocarcinoma in situ (AIS).

The absolute risk of disease (≥CIN2 and ≥CIN3) by APTIMA HPV 16 18/45 Genotype Assay result and APTIMA HPV Assay result are shown in the table below. The risk of ≥CIN2 in women with HPV types 16, 18, and/or 45 present was 29.1% compared to 14.3% in women with one or more of the other 11 high-risk HPV types present and 2.2.% in women with no high-risk HPV types present.

ASC-US ≥ 21 Years Population: Absolute Risk of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay

APTIMA HPV Assay Result	AHPV-GT Assay Result	Interpretation	≥CIN2	≥CIN3
			Absolute Risk (95% CI)	Absolute Risk (95% CI)
Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	29.1 (34/117) (22.4, 36.0)	16.2 (19/117) (11.4, 21.1)
	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	35.7 (25/70) (26.1, 45.9)	20.0 (14/70) (12.6, 28.0)
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	15.9 (7/44) (7.2, 28.3)	9.1 (4/44) (2.9, 19.5)
	HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	66.7 (2/3) (15.2, 98.2)	33.3 (1/3) (1.8, 84.6)
	HPV 16/18/45 Neg	Other HR HPV Pos	14.3 (33/231) (10.9, 17.9)	4.3 (10/231) (2.4, 6.8)
	Pos or Neg	HR HPV Pos	19.3 (67/348) (17.1, 21.3)	8.3 (29/348) (6.9, 9.4)
Negative	HPV 16/18/45 Neg*	HR HPV Neg	2.2 (12/545) (1.2, 3.5)	0.7 (4/545) (0.2, 1.6)
		Prevalence	8.8% (79/893)	3.7% (33/893)

AHPV-GT = APTIMA HPV 16 18/45 Genotype Assay, HR = High-risk, Pos = Positive, Neg = Negative
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

The absolute risk of disease (≥CIN2 and ≥CIN3) by APTIMA HPV 16 18/45 Genotype Assay result and APTIMA HPV Assay result are shown by age group in the table below.

ASC-US ≥ 21 Years Population: Absolute Risk of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay by Age Group

	APTIMA HPV Assay Result	AHPV-GT Assay Result	Interpretation	Absolute Risk ≥CIN2	Absolute Risk ≥CIN3
				Absolute Risk (95% CI)	Absolute Risk (95% CI)
21 to 29 Years	Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	26.8 (19/71) (18.3, 35.7)	15.5 (11/71) (9.3, 21.8)
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	28.0 (14/50) (17.5, 39.6)	18.0 (9/50) (9.9, 26.9)
		HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	15.8 (3/19) (3.7, 36.3)	5.3 (1/19) (0.2, 22.5)
		HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	100 (2/2) (27.0, 100)	50.0 (1/2) (2.9, 97.1)
		HPV 16/18/45 Neg	Other HR HPV Pos	17.0 (25/147) (12.6, 21.5)	5.4 (8/147) (2.8, 8.5)
		Pos or Neg	HR HPV Pos	20.2 (44/218) (17.6, 22.5)	8.7 (19/218) (7.1, 9.8)
	Negative	HPV 16/18/45 Neg*	HR HPV Neg	3.6 (6/165) (1.5, 6.9)	0.6 (1/165) (0.0, 2.7)
			Prevalence	13.1% (50/383)	5.2% (20/383)
30 to 39 Years	Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	32.3 (10/31) (19.0, 45.9)	16.1 (5/31) (7.0, 25.4)
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	50.0 (7/14) (24.2, 74.2)	21.4 (3/14) (5.1, 41.6)
		HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	18.8 (3/16) (3.0, 40.6)	12.5 (2/16) (1.3, 30.8)
		HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	0 (0/1) (0.0, 93.5)	0 (0/1) (0.0, 93.3)
		Pos or Neg	HR HPV Pos	19.8 (17/86) (15.1, 23.9)	8.1 (7/86) (4.7, 10.3)
		HPV 16/18/45 Neg	Other HR HPV Pos	12.7 (7/55) (6.2, 20.5)	3.6 (2/55) (0.6, 9.1)
	Negative	HPV 16/18/45 Neg*	HR HPV Neg	1.2 (2/167) (0.2, 3.5)	0.6 (1/167) (0.0, 2.3)
			Prevalence	7.5% (19/253)	3.2% (8/253)
≥ 40 Years	Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	33.3 (5/15) (12.4, 55.0)	20.0 (3/15) (4.1, 36.0)
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	66.7 (4/6) (27.1, 93.5)	33.3 (2/6) (6.2, 69.2)
		HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	11.1 (1/9) (0.5, 39.7)	11.1 (1/9) (0.5, 37.1)
		HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	--- (0/0)	--- (0/0)
		HPV 16/18/45 Neg	Other HR HPV Pos	3.4 (1/29) (0.1, 14.0)	0 (0/29) (0.0, 8.2)
		Pos or Neg	HR HPV Pos	13.6 (6/44) (6.5, 20.6)	6.8 (3/44) (1.8, 11.4)
	Negative	HPV 16/18/45 Neg*	HR HPV Neg	1.9 (4/213) (0.6, 3.4)	0.9 (2/213) (0.1, 2.0)
			Prevalence	3.9% (10/257)	1.9% (5/257)

AHPV-GT = APTIMA HPV 16 18/45 Genotype Assay, HR = High-risk, Pos = Positive, Neg = Negative
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

The relative risk of disease for APTIMA HPV 16 18/45 Genotype Assay positive versus negative outcomes is shown in the table below. Women who had HPV types 16, 18, and/or 45 present were 13.2 times more likely to have ≥CIN2 and 22.1 times more likely to have ≥CIN3 than women with no high-risk HPV types present. Women who had HPV types 16, 18, and/or 45 present were 2.0 times more likely to have ≥CIN2 and 3.8 times more likely to have ≥CIN3 than women with one or more of the other 11 high-risk HPV types present.

ASC-US ≥ 21 Years Population: Relative Risk of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay

APTIMA Assay Result Interpretation*	≥CIN2	≥CIN3
	Relative Risk (95% CI)	Relative Risk (95% CI)
HPV 16 and/or 18/45 Positive vs HR HPV Negative	13.2 (7.0, 24.7)	22.1 (7.7, 63.8)
HPV 16 and/or 18/45 Positive vs Other HR HPV Positive	2.0 (1.3, 3.1)	3.8 (1.8, 7.8)
Other HR HPV Positive vs HR HPV Negative	6.5 (3.4, 12.3)	5.9 (1.9, 18.6)
HR HPV Positive vs HR HPV Negative	8.7 (4.8, 15.9)	11.4 (4.0, 32.0)
Prevalence	8.8% (79/893)	3.7% (33/893)

CI = Confidence Interval, HR = High-risk
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

The likelihood ratios (≥CIN2 and ≥CIN3) by the APTIMA HPV 16 18/45 Genotype Assay result are shown in the table below. HPV types 16, 18, and/or 45 were 4.2 times more likely to be present in a woman with ≥CIN2 and 5.1 times more likely to be present in a woman with ≥CIN3.

ASC-US ≥ 21 Years Population: Likelihood Ratios for ≥CIN2 and ≥CIN3 by Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay

	APTIMA Assay Result Interpretation*	Likelihood Ratio (95% CI)
≥CIN2	HPV 16 and/or 18/45 Positive	4.2 (3.0, 5.8)
	Other HR HPV Positive	1.7 (1.3, 2.3)
	HR HPV Negative	0.2 (0.1, 0.4)
≥CIN3	HPV 16 and/or 18/45 Positive	5.1 (3.4, 6.9)
	Other HR HPV Positive	1.2 (0.6, 1.9)
	HR HPV Negative	0.2 (0.1, 0.4)

CI = Confidence Interval, HR = High-risk
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

NILM ≥ 30 Years Population: APTIMA HPV 16 18/45 Genotype Assay Clinical Performance

In total, there were 540 evaluable women 30 years of age and older with NILM cytology results and APTIMA HPV Assay positive results whose referral cytology samples were eligible for testing with the APTIMA HPV 16 18/45 Genotype Assay. Of these, 25 women (18 attended colposcopy and seven did not attend colposcopy) did not have referral cytology sample volume available for testing in this study; after a missing values analysis, they were not included in the performance calculations. The 515 evaluable women had valid APTIMA HPV 16 18/45 Genotype Assay results. Of these, 317 attended colposcopy. Fifteen (15) women had ≥CIN2 and 10 had ≥CIN3; 283 women had normal/CIN1 histology; 19 women had undetermined disease status.

Of the 298 evaluable women with conclusive disease status and APTIMA HPV Assay positive results, 61 had APTIMA HPV 16 18/45 Genotype Assay positive results, indicating the presence of HPV 16 and/or HPV 18/45; 237 had negative results, indicating the presence of one or more of the other 11 high-risk HPV types. An additional 505 evaluable women 30 years of age and older with NILM cytology results and conclusive disease status had APTIMA HPV Assay negative results during the CLEAR trial. An APTIMA HPV Assay negative result indicates that none of the 14 high-risk HPV types are present and were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis. The results of the APTIMA HPV 16 18/45 Genotype Assay by APTIMA HPV Assay result and consensus histology review panel diagnosis are presented in the table below.

NILM ≥ 30 Years Population: Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay by Consensus Histology Review Panel Diagnosis

APTIMA HPV Assay Result	AHPV-GT Assay Result*	Interpretation	Consensus Histology Review Panel Diagnosis						
			Undetermined**	Normal	CIN1	CIN2	CIN3	Cancer	Total
Positive	HPV 16 Neg, HPV 18/45 Neg	Other HR HPV Pos	16	218	11	4	4	0	253
	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos	2	27	0	0	3	1	33
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos	1	26	1	1	0	2	31
	HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	0	0	0	0	0	0	0
		Total	19	271	12	5	7	3	317
Negative	HPV 16/18/45 Neg***	HR HPV Neg	25	483	17	4	1	0	530
		Total	44	754	29	9	8	3****	847

AHPV-GT = APTIMA HPV 16 18/45 Genotype Assay, HR = High-risk, Pos = Positive, Neg = Negative
*All samples had final valid results (upon initial testing or after resolution of initial invalids per procedure).
**44 women attended the colposcopy visit but a diagnosis could not be determined for the following reasons: consensus could not be reached due to inadequate specimens (n=28), no biopsies collected due to underlying factors (n=13), no biopsies collected or reviewed due to error (n=3).
***Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.
****Three women had adenocarcinoma in situ (AIS).

Of the 515 women with APTIMA HPV Assay positive results and APTIMA HPV 16 18/45 Genotype Assay results, 217 women had unverified (including undetermined) disease status (see table below). Of the 10,331 women with APTIMA HPV Assay negative results from the original CLEAR trial, 9,826 had unverified disease status. Because the study was designed such that only randomly selected women with negative results for both the APTIMA HPV Assay and the FDA-approved DNA test were referred to colposcopy, the proportion of women with unverified disease status was high in this group (96.6%). To adjust for this verification bias, a multiple imputation method was used to estimate the number of women with disease that would have been identified if all women had undergone colposcopy. Both verification-bias adjusted performance estimates and unadjusted performance estimates based on the 803 women with verified disease status are presented.

NILM ≥ 30 Years Population: Classification of Evaluable NILM Women by APTIMA HPV Assay, APTIMA HPV 16 18/45 Genotype Assay, HPV DNA Test Results, Disease Status (≥CIN2 and ≥CIN3), and Disease Verification Status

APTIMA HPV Assay Result*	AHPV-GT Assay Result*	HPV DNA Test	Total Women	Verified Disease Status: ≥CIN2		Verified Disease Status: ≥CIN3		Unverified Disease Status
				Diseased Women (≥CIN2)	Non-Diseased Women (<CIN2)	Diseased Women (≥CIN3)	Non-Diseased Women (<CIN3)	Women with Unknown Disease Status (% Unknown)
Positive	Positive	Positive	83	6	48	5	49	29 (34.9%)
	Positive	Negative	9	1	5	1	5	3 (33.3%)
	Positive	No Result**	2	0	1	0	1	1 (50.0%)
	Negative	Positive	271	7	171	4	174	93 (34.3%)
	Negative	Negative	137	1	52	0	53	84 (61.3%)
	Negative	No Result**	13	0	6	0	6	7 (53.8%)
		Total	515	15	283	10	288	217 (42.1%)
Negative	N/A***	Positive	306	3	178	1	180	125 (40.8%)
	N/A***	Negative	9,420	1	322	0	323	9,097 (96.6%)
	N/A***	No Result**	605	1	0	0	1	604 (99.8%)
		Total	10,846	20	783	11	792	10,043 (92.6%)

AHPV-GT = APTIMA HPV 16 18/45 Genotype Assay, N/A = Not Applicable
*All samples had final valid results (upon initial testing or after resolution of initial invalids per procedure).
**620 women with APTIMA HPV Assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.
***Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

The adjusted absolute risks of disease (≥CIN2 and ≥CIN3) by APTIMA HPV 16 18/45 Genotype Assay result and APTIMA HPV Assay result are shown in the table below. The risk of ≥CIN2 in women with HPV types 16, 18, and/or 45 present was 12.6% compared to 3.4% in women with one or more of the other 11 high-risk HPV types present and 0.6% in women with no high-risk HPV types present.

NILM ≥ 30 Years Population: Absolute Risk of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay (Verification-Bias Adjusted Estimates)

APTIMA HPV Assay Result	AHPV-GT Assay Result	Interpretation	≥CIN2	≥CIN3
			Absolute Risk (95% CI)	Absolute Risk (95% CI)
Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	12.6 (3.7, 21.4)	9.5 (2.1, 16.8)
	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	14.5 (2.1, 26.9)	12.1 (0.7, 23.4)
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	10.7 (0.0, 22.5)	6.9 (0.0, 16.2)
	HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	N/A	N/A
	HPV 16/18/45 Neg	Other HR HPV Pos	3.4 (1.2, 5.6)	1.8 (0.1, 3.5)
	Pos or Neg	HR HPV Pos	5.0 (2.6, 7.5)	3.2 (1.3, 5.2)
Negative	HPV 16/18/45 Neg*	HR HPV Neg	0.6 (0.1, 1.2)	0.4 (0.0, 0.7)
		Prevalence	0.9%	0.5%

AHPV-GT = APTIMA HPV 16 18/45 Genotype Assay, HR = High-risk, Pos = Positive, Neg = Negative, N/A = Not Applicable
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

The unadjusted absolute risks of disease (≥CIN2 and ≥CIN3) by APTIMA HPV 16 18/45 Genotype Assay result and APTIMA HPV Assay result are shown overall in the table below.

NILM ≥ 30 Years Population: Absolute Risk of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay (Unadjusted Estimates)

APTIMA HPV Assay Result	AHPV-GT Assay Result	Interpretation	≥CIN2	≥CIN3
			Absolute Risk (95% CI)	Absolute Risk (95% CI)
Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	11.5 (7/61) (5.4, 18.9)	9.8 (6/61) (4.6, 15.2)
	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	12.9 (4/31) (4.0, 26.0)	12.9 (4/31) (4.3, 23.8)
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	10.0 (3/30) (2.4, 23.0)	6.7 (2/30) (0.8, 17.7)
	HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	N/A (0/0)	N/A (0/0)
	HPV 16/18/45 Neg	Other HR HPV Pos	3.4 (8/237) (1.7, 5.3)	1.7 (4/237) (0.6, 3.2)
	Pos or Neg	HR HPV Pos	5.0 (15/298) (3.6, 6.2)	3.4 (10/298) (2.3, 3.9)
Negative	HPV 16/18/45 Neg*	HR HPV Neg	1.0 (5/505) (0.4, 1.9)	0.2 (1/505) (0.0, 0.9)
		Prevalence	2.5% (20/803)	1.4% (11/803)

AHPV-GT = APTIMA HPV 16 18/45 Genotype Assay, HR = High-risk, Pos = Positive, Neg = Negative, N/A = Not Applicable
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

The unadjusted absolute risks of disease (≥CIN2 and ≥CIN3) by APTIMA HPV 16 18/45 Genotype Assay result and APTIMA HPV Assay result are shown by age group in the table below.

NILM ≥ 30 Years Population: Absolute Risk of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay by Age Group (Unadjusted Estimates)

	APTIMA HPV Assay Result	AHPV-GT Assay Result	Interpretation	≥CIN2	≥CIN3
				Absolute Risk (95% CI)	Absolute Risk (95% CI)
30 to 39 Years	Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	8.8 (3/34) (2.2, 17.8)	5.9 (2/34) (1.0, 13.3)
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	0 (0/17) (0.0, 15.5)	0 (0/17) (0.0, 14.3)
		HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	17.6 (3/17) (3.2, 35.4)	11.8 (2/17) (1.3, 27.0)
		HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	N/A (0/0)	N/A (0/0)
		HPV 16/18/45 Neg	Other HR HPV Pos	4.0 (5/124) (1.7, 6.2)	2.4 (3/124) (0.7, 4.2)
		Pos or Neg	HR HPV Pos	5.1 (8/158) (3.2, 6.1)	3.2 (5/158) (1.5, 4.0)
	Negative	HPV 16/18/45 Neg*	HR HPV Neg	0.5 (1/217) (0.0, 1.9)	0.5 (1/217) (0.0, 1.7)
			Prevalence	2.4% (9/375)	1.6% (6/375)
≥ 40 Years	Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	14.8 (4/27) (4.7, 27.3)	14.8 (4/27) (5.1, 22.8)
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	28.6 (4/14) (6.3, 50.7)	28.6 (4/14) (6.4, 46.5)
		HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	0 (0/13) (0.0, 20.1)	0 (0/13) (0.0, 17.1)
		HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	N/A (0/0)	N/A (0/0)
		HPV 16/18/45 Neg	Other HR HPV Pos	2.7 (3/113) (0.7, 5.8)	0.9 (1/113) (0.0, 3.1)
		Pos or Neg	HR HPV Pos	5.0 (7/140) (2.6, 7.0)	3.6 (5/140) (1.9, 4.2)
	Negative	HPV 16/18/45 Neg*	HR HPV Neg	1.4 (4/288) (0.5, 2.5)	0 (0/288) (0.0, 0.8)
			Prevalence	2.6% (11/428)	1.2% (5/428)

AHPV-GT = APTIMA HPV 16 18/45 Genotype Assay, HR = High-risk, Pos = Positive, Neg = Negative, N/A = Not Applicable
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

The relative risk of disease for APTIMA 16 18/45 Genotype Assay positive versus negative outcomes are shown in the tables below (verification-bias adjusted and unadjusted). Women who had HPV types 16, 18, and/or 45 present were 20.9 times more likely to have ≥CIN2 and 29.4 times more likely to have ≥CIN3 than women with no high-risk HPV types present. Women who had HPV types 16, 18, and/or 45 present were 3.7 times more likely to have ≥CIN2 and 5.3 times more likely to have ≥CIN3 than women with one or more of the other 11 high-risk HPV types present.

NILM ≥ 30 Years Population: Relative Risk of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay (Verification-Bias Adjusted Estimates)

APTIMA Assay Test Interpretation*	≥CIN2	≥CIN3
	Relative Risk (95% CI)	Relative Risk (95% CI)
HPV 16 and/or 18/45 Pos vs HR HPV Neg	20.9 (6.3, 69.3)	29.4 (7.2, 120.8)
HPV 16 and/or 18/45 Pos vs Other HR HPV Pos	3.7 (1.5, 9.5)	5.3 (1.5, 18.2)
Other HR HPV Pos vs HR HPV Neg	5.6 (1.8, 17.7)	5.6 (1.2, 26.0)
HR HPV Pos vs HR HPV Neg	8.5 (2.9, 24.8)	10.1 (2.7, 38.2)
Prevalence	0.9%	0.5%

CI = Confidence Interval, HR = High-risk, Pos = Positive, Neg = Negative
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

NILM ≥ 30 Years Population: Relative Risk of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay (Unadjusted Estimates)

APTIMA Assay Test Interpretation*	≥CIN2	≥CIN3
	Relative Risk (95% CI)	Relative Risk (95% CI)
HPV 16 and/or 18/45 Pos vs HR HPV Neg	11.6 (3.8, 35.4)	49.7 (6.1, 406)
HPV 16 and/or 18/45 Pos vs Other HR HPV Pos	3.4 (1.3, 9.0)	5.8 (1.7, 20.0)
Other HR HPV Pos vs HR HPV Neg	3.4 (1.1, 10.3)	8.5 (1.0, 75.8)
HR HPV Pos vs HR HPV Neg	5.1 (1.9, 13.8)	16.9 (2.2, 132)
Prevalence	2.5% (20/803)	1.4% (11/803)

CI = Confidence Interval, HR = High-risk, Pos = Positive, Neg = Negative
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

The likelihood ratios (≥CIN2 and ≥CIN3) by the APTIMA 16 18/45 Genotype Assay result are shown in the tables below (verification-bias adjusted and unadjusted). HPV types 16, 18, and/ or 45 were 17.1 times more likely to be present in a woman with ≥CIN2 and 21.9 times more likely to be present in a woman with ≥CIN3.

NILM ≥ 30 Years Population: Likelihood Ratios for ≥CIN2 and ≥CIN3 by Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay (Verification-Bias Adjusted Estimates)

	APTIMA Assay Result Interpretation*	Likelihood Ratio (95% CI)
≥CIN2	HPV 16 and/or 18/45 Positive	17.1 (6.2, 46.9)
	Other HR HPV Positive	4.2 (1.7, 10.1)
	HR HPV Negative	0.7 (0.5, 1.0)
≥CIN3	HPV 16 and/or 18/45 Positive	21.9 (7.3, 65.2)
	Other HR HPV Positive	3.8 (1.2, 12.6)
	HR HPV Negative	0.7 (0.4, 1.1)

CI = Confidence Interval, HR = High-risk
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

NILM ≥ 30 Years Population: Likelihood Ratios and for ≥CIN2 and ≥CIN3 by Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay (Unadjusted Estimates)

	APTIMA Assay Result Interpretation*	Likelihood Ratio (95% CI)
≥CIN2	HPV 16 and/or 18/45 Positive	5.1 (2.3, 9.1)
	Other HR HPV Positive	1.4 (0.7, 2.2)
	HR HPV Negative	0.4 (0.1, 0.7)
≥CIN3	HPV 16 and/or 18/45 Positive	7.9 (3.5, 12.9)
	Other HR HPV Positive	1.2 (0.4, 2.3)
	HR HPV Negative	0.1 (0.0, 0.6)

CI = Confidence Interval, HR = High-risk
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

Subgroup Analysis

The table below presents clinical performance for the APTIMA 16 18/45 Genotype Assay for the ASC-US population by testing site

ASC-US ≥ 21 Years Population: Absolute Risk of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay by Testing Site

Testing Site*	APTIMA HPV Assay Result	AHPV-GT Assay Result	Interpretation	Absolute Risk ≥CIN2	Absolute Risk ≥CIN3
				Absolute Risk (95% CI)	Absolute Risk (95% CI)
1	Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	29.3 (12/41) (18.2, 41.0)	12.2 (5/41) (4.9, 20.0)
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	29.6 (8/27) (14.2, 45.8)	11.1 (3/27) (2.9, 23.0)
		HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	16.7 (2/12) (1.8, 43.1)	8.3 (1/12) (0.3, 31.2)
		HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	100 (2/2) (27.1, 100)	50.0 (1/2) (2.9, 97.0)
		HPV 16/18/45 Neg	Other HR HPV Pos	14.7 (11/75) (8.7, 21.2)	5.3 (4/75) (1.8, 9.6)
		Pos or Neg	HR HPV Pos	19.8 (23/116) (15.9, 23.4)	7.8 (9/116) (5.1, 9.4)
	Negative**	HPV 16/18/45 Neg	HR HPV Neg	1.6 (3/183) (0.4, 4.0)	0.5 (1/183) (0.0, 2.2)
			Prevalence	8.7% (26/299)	3.3% (10/299)
2	Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	34.1 (14/41) (21.9, 46.4)	22.0 (9/41) (12.5, 31.5)
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	45.5 (10/22) (25.7, 64.2)	27.3 (6/22) (11.1, 44.1)
		HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	21.1 (4/19) (6.5, 41.6)	15.8 (3/19) (3.7, 34.2)
		HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	--- (0/0)	--- (0/0)
		HPV 16/18/45 Neg	Other HR HPV Pos	13.4 (11/82) (7.8, 19.8)	4.9 (4/82) (1.6, 9.7)
		Pos or Neg	HR HPV Pos	20.3 (25/123) (16.3, 24.0)	10.6 (13/123) (7.6, 12.7)
	Negative**	HPV 16/18/45 Neg	HR HPV Neg	2.8 (5/180) (1.0, 5.4)	1.1 (2/180) (0.2, 3.1)
			Prevalence	9.9% (30/303)	5.0% (15/303)
3	Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	22.9 (8/35) (11.4, 35.7)	14.3 (5/35) (6.1, 22.5)
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	33.3 (7/21) (15.4, 52.2)	23.8 (5/21) (9.0, 37.9)
		HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	7.7 (1/13) (0.3, 31.3)	0 (0/13) (0.0, 19.2)
		HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	0 (0/1) (0.0, 93.5)	0 (0/1) (0.0, 93.3)
		HPV 16/18/45 Neg	Other HR HPV Pos	14.9 (11/74) (8.9, 21.1)	2.7 (2/74) (0.4, 6.8)
		Pos or Neg	HR HPV Pos	17.4 (19/109) (13.3, 21.1)	6.4 (7/109) (3.8, 8.0)
	Negative**	HPV 16/18/45 Neg	HR HPV Neg	2.2 (4/182) (0.7, 4.6)	0.5 (1/182) (0.0, 2.1)
			Prevalence	7.9% (23/291)	2.7% (8/291)

AHPV-GT = APTIMA HPV 16 18/45 Genotype Assay, HR = High-risk, Pos = Positive, Neg = Negative

* The testing site reflects where the APTIMA HPV 16 18/45 Genotype Assay was tested for the subjects with positive Aptima HPV Assay results,. For the Aptima HPV Assay negative subjects, the testing site reflects where the Aptima HPV Assay was tested.

**Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

Agreement with Reverse Transcription-PCR Sequencing

The analytical performance of the APTIMA HPV 16 18/45 Genotype Assay for detection of target was assessed against an in-house validated reverse transcription-polymerase chain reaction (RT-PCR) sequencing test specific for E6/E7 mRNA from the same 14 HR HPV types detected by the APTIMA HPV Assay. Sequencing was performed by an external commercial laboratory.

Cervical specimens collected from the ASC-US and NILM populations of the CLEAR trial from women with APTIMA HPV Assay positive results were tested with the RT-PCR sequencing test and compared to the APTIMA HPV 16 18/45 Genotype Assay results. In total, 859 samples were tested: 354 from the ASC-US population and 505 from the NILM population.

For the ASC-US and NILM populations, APTIMA HPV 16 18/45 Genotype Assay results by RT-PCR sequencing test results are shown in the tables below.

ASC-US ≥ 21 Years Population: Comparison of APTIMA HPV 16 18/45 Genotype Assay and RT-PCR Sequencing Test Results Including Only Samples With APTIMA HPV Assay Positive Results

	RT-PCR Sequencing Test Results											
APTIMA HPV-GT Assay Result	No HR Type	One HR Type				Two HR Types				>2 HR Types		Ind
		16	18	45	Other HR	16 & Other	18 & Other	45 & Other	2 Other HR	≥1 of 16/18/45 Present	Only Other HR Present	
16+, 18/45-	27	27	0	0	6	7	0	0	1	3	0	0
16-, 18/45+	4	0	17	9	4	0	4	4	1	4	0	0
16+, 18/45+	0	0	1	0	0	1	0	0	0	1	0	0
16-, 18/45-	76	0	1	1	128	0	2	1	16	0	6	2
Total	107	27	19	10	138	8	6	5	18	8	6	2

HPV-GT = APTIMA HPV 16 18/45 Genotype Assay, + = Positive, - = Negative, HR = high risk, Ind = indeterminate; unable to determine positivity for types 16/18/45 due to invalid RT-PCR sequencing test results.
Columns with all zeros are not shown

NILM ≥30 Years Population: Comparison of APTIMA HPV 16 18/45 Genotype Assay and RT-PCR Sequencing Test Results Including Only Samples With APTIMA HPV Assay Positive Results

	RT-PCR Sequencing Test Results										
APTIMA HPV-GT Assay Result	No HR Type	One HR Type				Two HR Types				>2 HR Types	Ind
		16	18	45	Other HR	16 & Other	18 & Other	45 & Other	2 Other HR	≥1 of 16/18/45 Present	
16+, 18/45-	24	19	0	0	2	1	0	0	0	0	0
16-, 18/45+	7	0	18	12	1	0	2	5	0	1	0
16+, 18/45+	0	0	0	0	0	0	0	0	0	0	0
16-, 18/45-	251	0	2	4	148	1	0	0	4	0	3

Total	282	19	20	16	151	2	2	5	4	1	3

HPV-GT = APTIMA HPV 16 18/45 Genotype Assay, + = Positive, - = Negative, HR = high risk , Ind = indeterminate; unable to determine positivity for types 16/18/45 due to invalid RT-PCR sequencing test results.
Columns with all zeros are not shown

Positive and negative percent agreements with RT-PCR sequencing test results for the ASC-US and NILM populations are shown in the tables below.

ASC-US ≥ 21 Years Population: Comparison of APTIMA HPV 16 18/45 Genotype Assay and RT-PCR Sequencing Test Results Including Only Samples With APTIMA HPV Assay Positive Results

RT-PCR Sequencing Test Results			
APTIMA HPV-GT Assay Result		**16/18/45-**	
	16/18/45+	**Other HR+**	**HR-**
16/18/45+	78	12	31
16/18/45-	5	150	76
Total	83	162	107
Positive Percent Agreement: 94.0 (78/83) (95% CI: 86.7, 97.4)			
Negative Percent Agreement: 92.6 (150/162) (95% CI: 87.5, 95.7)			

CI = Confidence Interval, HPV-GT = APTIMA HPV 16 18/45 Genotype Assay, + = Positive, - = Negative, HR = high risk

NILM ≥30 Years Population: Comparison of APTIMA HPV 16 18/45 Genotype Assay and RT-PCR Sequencing Test Results Including Only Samples With APTIMA HPV Assay Positive Results

RT-PCR Sequencing Test Results			
APTIMA HPV-GT Assay Result		**16/18/45-**	
	16/18/45+	**Other HR+**	**HR-**
16/18/45+	58	3	31
16/18/45-	7	152	251
Total	65	155	282
Positive Percent Agreement: 89.2 (58/65) (95% CI: 79.4, 94.7)			
Negative Percent Agreement: 98.1 (152/155) (95% CI: 94.5, 99.3)			

CI = Confidence Interval, HPV-GT = APTIMA HPV 16 18/45 Genotype Assay, + = Positive, - = Negative, HR = high risk

Expected Results: Prevalence of High-Risk HPV mRNA

The prevalence of high-risk HPV infection varies widely and is influenced by several factors, of which age is the greatest contributor. Many studies have investigated HPV prevalence as determined by the detection of HPV DNA, however few studies report prevalence based on detection of HPV oncogenic mRNA. Women from a variety of clinical sites (n=18) representing a wide geographic distribution and a diverse population (10 states within the United States) were enrolled in a prospective clinical study known as the CLEAR trial to evaluate the APTIMA HPV Assay, which detects 14 high-risk HPV types. Samples from women in the CLEAR trial with APTIMA HPV Assay positive results were evaluated at three testing sites with the APTIMA HPV 16 18/45 Genotype Assay in a separate clinical study. The prevalence of HPV 16, 18/45, as well as the remaining 11 high-risk HPV types observed in the clinical study, based on results of testing with the APTIMA HPV Assay and the APTIMA HPV 16 18/45 Genotype Assay, was categorized overall, by age group, and by testing site. Results are shown in the table below for the atypical squamous cells of undetermined significance (ASC-US) and the negative for intraepithelial lesion or malignancy (NILM) populations.

High-risk HPV mRNA Prevalence in Populations by Age Group, Testing Site, and All Combined

	Positivity Rate % (x/n)							
	ASC-US Population (≥ 21 Years)				NILM Population (≥ 30 Years)			
	HPV 16 Pos	HPV 18/45 Pos	HPV 16 & 18/45 Pos	11 Other HR* Pos	HPV 16 Pos	HPV 18/45 Pos	HPV 16 & 18/45 Pos	11 Other HR* Pos
All	7.8 (71/912)	5.2 (47/912)	0.3 (3/912)	25.5 (233/912)	0.4 (47/10,846)	0.4 (47/10,846)	0 (0/10,846)	3.9 (421/10,846)
Age Group (years)								
21 to 29	13.2 (51/386)	4.9 (19/386)	0.5 (2/386)	38.3 (148/386)	N/A	N/A	N/A	N/A
30 to 39	5.4 (14/257)	7.0 (18/257)	0.4 (1/257)	21.8 (56/257)	0.7 (30/4,188)	0.6 (27/4,188)	0 (0/4,188)	5.3 (221/4,188)
≥ 40	2.2 (6/269)	3.7 (10/269)	0 (0/269)	10.8 (29/269)	0.3 (17/6,658)	0.3 (20/6,658)	0 (0/6,658)	3.0 (200/6,658)
Testing Site								
1	9.0 (27/301)	4.3 (13/301)	0.7 (2/301)	24.9 (75/301)	0.4 (13/3,666)	0.5 (18/3,666)	0 (0/3,666)	3.8 (141/3,666)
2	7.4 (23/310)	6.1 (19/310)	0 (0/310)	26.5 (82/310)	0.5 (18/3,671)	0.5 (17/3,671)	0 (0/3,671)	3.7 (136/3,671)
3	7.0 (21/301)	5.0 (15/301)	0.3 (1/301)	25.2 (76/301)	0.5 (16/3,509)	0.3 (12/3,509)	0 (0/3,509)	4.1 (144/3,509)

N/A = Not Applicable, HR = High-risk, Pos = Positive

* HPV types 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and 68

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Microbiology Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The effectiveness of the APTIMA HPV 16 18/45 Genotype Assay has been demonstrated for use in conjunction with cervical cytology in the following patient populations. The test may be used in women 30 years and older to adjunctively screen to assess the presence or absence of high-risk human papillomavirus (HPV) genotypes 16, 18, and/or 45 in women with APTIMA HPV Assay positive results. Additionally, a reasonable determination of effectiveness of the APTIMA HPV Assay for use in testing women ≥21 years with ASC-US cervical cytology results and APTIMA HPV Assay positive results has been demonstrated. The results of this test, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.

B. Safety Conclusions

The risks of the device are based on data collected in a clinical study conducted to support PMA approval as described above. False positive results may lead to morbidity associated with unnecessary colposcopy. False negative results may lead to delay in evaluation and treatment for CIN. Based on the results of the analytical and clinical studies, the APTIMA HPV 16 18/45 Genotype Assay, when used according to the provided directions and together with the physician's interpretation of cytology results, other risk factors, and professional guidelines, should be safe and pose minimal risk to the patient due to false test results.

C. Benefit-Risk Conclusions

The probable benefits of the device are based on data collected in clinical studies conducted to support PMA approval as described above. The benefit of using the APTIMA HPV 16 18/45 Genotype Assay has been demonstrated in conjunction with cervical cytology in the following patient populations: In patients 21 years and older with atypical squamous cells of undetermined significance (ASC-US) cervical cytology results as well as in women 30 years and older, the APTIMA HPV 16 18/45 Genotype Assay can be used to test samples from women with APTIMA HPV Assay positive results to assess the presence or absence of high-risk HPV genotypes 16, 18, and/or 45. Patients with positive results have a higher probability of ≥CIN2; patients with negative results have a lower probability of ≥CIN2. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management. Benefit risk considerations were similar for a previously FDA approved device.

In conclusion, given the available information above, the data support that for the approved intended use, the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. The data from the nonclinical studies demonstrated acceptable analytical sensitivity, precision, and analytical specificity of the APTIMA HPV 16 18/45 Genotype Assay when used according to the instructions for use, the warnings and precautions, and limitations sections of the labeling. The clinical studies and the statistical analysis of clinical data in this application has shown that the assay is safe and effective for its approved indications when used according to the directions for use in the labeling.

XIII. CDRH DECISION

CDRH issued an approval order on October 12, 2012. The final conditions of approval are described in the approval order.

The applicant's manufacturing facilities were inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820) on June 15, 2012.

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.

XV. REFERENCES

[1] http://www.accessdata.fda.gov/cdrh_docs/pdf10/P100042b.pdf

[2] Debbie Saslow et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology Screening Guidelines for the Prevention and Early Detection of Cervical Cancer. *Am J Clin Pathol* 2012;137:516-542.